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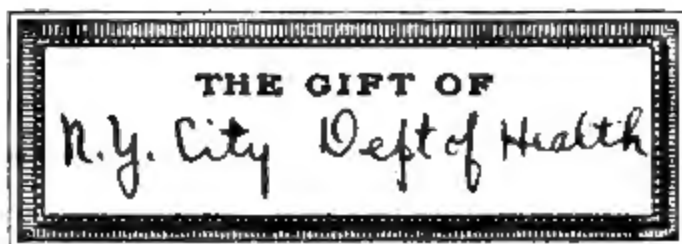
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COLLECTED STUDIES

FROM THE

RESEARCH LABORATORY

DEPARTMENT OF HEALTH

NEW YORK CITY

Dr. W. H. PARK, Director.

VOLUME I.

1905

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NEGRI BODIES, WITH SPECIAL REFERENCE TO DIAGNOSIS IN SUSPECTED RABIES.

BY ANNA W. WILLIAMS, M. D.,

*Assistant Director, Research Laboratory.**

The history of the findings in hydrophobia is rather instructive. Up to 1903, notwithstanding much painstaking work on the lesion in this disease by many of our most eminent pathologists, certain bodies described then for the first time by Negri (possibly seen before by one other recording observer), were entirely overlooked. And what makes it more interesting is the fact that these bodies, with the methods now in use, come out with such startling distinctness and individuality that even the beginner in studies on the nervous system cannot miss seeing them.

In the research laboratory of the Department of Health Dr. Poor has corroborated the work of others in regard to the presence and specific nature of these bodies in fixed and stained sections from the nerve centers in cases of rabies, and, for some time, has used the section method for diagnosis. By this method, however, it is impossible to make the diagnosis before 24 hours, and no one, so far as known, has published a much shorter way of demonstrating the bodies satisfactorily.

In connection with a report made by Dr. Poor before the New York Pathological Society in 1904, the writer demonstrated a smear from hydrophobia brain tissue containing these Negri bodies and recommended the smear method for rapid diagnosis. Smears were made and studied at that time at the suggestion of Dr. Ewing who, as we all know, had obtained such good results by this method in his "Studies on Ganglion Cells."

By the technic then employed, though the Negri bodies were brought out clearly, they were delicately stained and their differentiation from the surrounding tissues, especially from the red blood cells was somewhat difficult.

Recently the work has been taken up by me again, and it has been found that by slightly improved technic and a different stain, the

* Read at the New York Pathological Society December, 1905.

"bodies," in most instances, if not in all, are brought out more distinctly and more characteristically, and can be identified in a much shorter time and by a much simpler and less expensive technic than by any method so far published.

The work has been controlled on one side by section cuttings and animal inoculations and, on the other side, by smears from the central nervous system of normal animals and of animals that have died from tetanus, diphtheria and meningitis; and it seems reasonable to make the positive statement that the bodies seen in smears as well as those seen in sections are specific to hydrophobia.

Further, the smear work seems to have brought out more clearly than the section work that these bodies are not degeneration products, that is that they have an individuality distinct from the nervous tissue. This is shown by the fact that they are definitely and regularly structured according to the age of the lesion and that the structure and staining qualities are analogous to those of certain known protozoa, therefore it makes it practically certain in my opinion that they are living organisms belonging to the group of protozoa and that they are the active cause of hydrophobia.

The technic is as follows: The nerve tissue is obtained as soon as possible after the death of the animal (though smears made as late as 13 days after death when the tissue had been kept in the ice-box have shown bodies stained well enough for diagnosis), and smears may be made in any way that will get the tissue quickly and evenly on the slide. However, I have found that the best results can be obtained by the following method:

The small bit of nerve tissue required for the examination is cut out with the point of a small scalpel or with a small, sharp scissors and placed upon a glass slide. A cover slip is put over the piece and pressed upon it gently until it is well spread out, then, with the finger still pressing lightly, the cover slip is moved along to the end of the slide. It moves very easily and makes a thin more or less evenly spread smear.

The smears are allowed to dry in the air and are fixed either in Zenker's fluid or in methyl alcohol.

The two staining methods which have been principally used are Mallory's eosin-methylene-blue method and Giemsa's azur-eosin method. A comparison between the results obtained by the use of these two stains is most instructive. The eosin-methylene-blue method, if the decolorization is carried to a marked extent, shows the protoplasm of the bodies staining with eosin, while the central bodies and granules are a dark blue. When the decolorization is not carried so far the cytoplasm of the bodies appear more of a magenta, showing that they are not purely acidophile.

In sections the apparent acidophile character of the bodies is even more marked than in the smears, hence observers have always spoken of them as acidophile, and since protozoa are known to be basophile, opponents of the protozoan theory of their nature have considered this a strong point on their side.

The Giemsa stain with the smears, however, has shown that they are really more basophile than acidophile in character and that the central body takes up the pure nuclear stain—the azur.

The bodies vary in their stained appearance according to the time stained, the amount of decolorization, the thickness of the smear, the age of the lesion and the length of time after death. In the stage of the disease that is usually found in the so-called "street rabies," that is in the stage that oftenest reaches us for diagnosis, most of the bodies, according to the Giemsa method of staining with the stain left on for one hour, stain a rather dark robin's egg blue, the granules and central bodies purple. With more decolorization, the protoplasm is a clearer blue and the chromatin masses red. Many of the bodies by this method of staining show a double contour or membrane-like periphery which may be an artifact as it is more apparent in bodies within the cells and in the thicker parts of the smears. For the purpose of diagnosis the bodies may be stained sufficiently characteristically with concentrated Giemsa in 10 minutes.

The finer structure of these bodies will not be spoken of in detail at present, suffice it to say that the small rounded forms show a chromatin staining ring-shaped central body, the small oval forms may show two or three such bodies, some of the larger rounded forms show chromatin bodies arranged more or less regularly around the periphery as

well as in the center. Some of the large oval forms show a chromatin rounded body nearer one end with small chromatin granules irregularly scattered throughout the rest of the body.

The staining characteristics together with the fact that the morphology is so characteristic, makes it practically certain, as I have said, that the bodies are protozoa, and their site, time of appearance, numbers and absence from other diseases make it as certain that they are the cause of hydrophobia.

RECENT STUDIES IN THE DIAGNOSIS OF RABIES.

BY DANIEL W. POOR, M. D.,

*Assistant Bacteriologist Research Laboratory of the
Department of Health.*

It is obvious to those who have to deal with people bitten by animals supposedly rabid, that a quick method for the diagnosis of this disease in the animal is most desirable. The length of time consumed by the inoculation test, with its consequent suspense and worry to the patient, together with the resulting delay in starting treatment, and further the occasional failure of the test through premature death of the animals from septicemia, are all reasons for stimulating work on the histological methods of diagnosis.

Since the time of Pasteur, work has been done along this line, and it may be of some interest to summarize very briefly the results without going extensively into the technicalities of the subject. It is obvious that a satisfactory method of diagnosis must fulfil, as nearly as possible, the following conditions. First, the lesions should be characteristic of the disease; second, they should appear early in the disease as well as late; third, the technique should not be so difficult and exacting as to render its accomplishment frequently impossible under the conditions which we have to meet practically, and further the lesions should be sufficiently definite to form the basis of a positive opinion. Lastly, it is desirable that the microscopical picture be as little as possible affected by the changes produced by post-mortem decomposition and freezing.

Some years ago Babes described lesions which he considered characteristic of rabies, the essential points of which were, a collection of embryonal cells surrounding the central canal of the cord, and the ganglion cells, particularly in the medulla. The ganglion cells degenerate—chromatolysis being especially characteristic—and their spaces are occupied by the embryonal cells which constitute the so-called “rabid tubercle.” From what can be learned, this method has not been in general use extensively, owing to the fact that these lesions are not constantly present in rabies, and that they may also be found in other conditions.

Later, Van Gehuchten and Nelis described changes in the sympathetic ganglia, the intervertebral ganglia, and in the plexiform ganglia of the pneumogastric nerve. In these locations the nerve cells lie in capsules lined with endothelial cells. The changes said to be characteristic of rabies are the atrophy and destruction of the nerve cells brought about by the new formed cells from the capsule, which finally occupy the entire capsule.

Ravanel, who has used this method of diagnosis, considered that the changes in the intervertebral ganglia are more constant than those in the medulla. He has reported a series of twenty-eight cases, including eleven dogs, one cow and one horse, all having street rabies, and fifteen rabbits inoculated from these animals. In all except the horse positive changes were found, although in one of the cases they were very slight. In twenty-one cases examined for the lesions described by Babes, seventeen showed characteristic lesions. In two only chromatolysis of the cells was found, and in two no lesions were seen. Similar lesions to those of Van Gehuchten have been found in the ganglia by Crocq in one case, and by Van Gehuchten in three cases of diphtheria. Four cases in man have been reported as having lesions identical with those of rabies, including epithelioma of the Gasserian ganglion, acute ascending paralysis of the cord, and carcinoma of the rectum. It is also stated that the lesions of Van Gehuchten and Nelis are not well marked in the early stages of the disease. It seems, then, that the rabid tubercles of Babes, while frequently found, are not constant. The various phases of cell degeneration, such as chromatolysis, granular degeneration, loss of nerve processes, and the various stages of cell destruction are in-

interesting to observe in connection with the pathology of the disease, but they can scarcely be made the basis of diagnosis, since they may be caused by other poisons. Further, owing to the great liability of the nervous system to rapidly undergo post-mortem change, together with the frequent production in it of artefacts from rough handling and improper fixatives, we have still further reasons for considering these minute changes as unreliable for diagnosis under the conditions which we have to meet practically.

Assuming that the lesions of Van Gehuchten are more constantly found in rabies, the great difficulty of the dissection of the intervertebral and plexiform ganglia makes this method undesirable, to my mind, as a routine method of diagnosis.

More recently, in 1903, Negri, of the University of Pavia, has made important observations on the pathology of rabies, describing minute bodies, since called Negri bodies, or corpuscles, which are found chiefly as cell inclusions in the Purkinje cells of the cerebellum and in the large ganglion cells in the region of ammons horn. Negri and some others believe these bodies to be the causative factor in the disease, and classify them among the protozoa. While not attracting much attention at first, these bodies have been studied and reported on latterly by a large number of observers, and they have become the basis for a method of diagnosis in a number of laboratories. Briefly described, these bodies may be said to be minute structures, varying in size from one to twenty-three microns in diameter. The shape is round or oval, but may be quite irregular. The staining reaction is eosinophile. In structure they may be homogeneous, ring formed, or vacuolated. They may contain irregularly grouped granules, or they may present a certain definite structure, namely, that of a mass of protoplasm containing one or more nuclear-like bodies surrounded by circular unstained areas. This structure has also been demonstrated by Dr. Williams of this laboratory in stained smear preparations. A preliminary report of her work will be found on page 3. They have been seen in the hanging drop as well as in stained preparation. They are said to preserve their form in spite of putrefaction of the brain, after prolonged immersion in glycerin, and after several days' drying. From personal experience, I can state that they may be found after such marked post-mortem change has oc-

curred as to render the nerve cells themselves unfit for the histological study of their own structure. Further, I have found them easily in a brain packed in ice for forty-eight hours and which had become completely frozen. These bodies are not found in the salivary glands.

As to the nature of these cell inclusions, suffice it to say that, while some believe them to be protozoa, others hold that they represent the degeneration of red blood cells, or of some of the cellular elements of the central nervous system. The following series will give an idea as to the frequency with which these bodies have been found. Taking the combined statistics of six European laboratories, we have a total of 550 observations. In 344 cases the lesions described by Negri were found. In every one of these cases the animal was proved to have rabies by the biological test. In 206 cases the lesions were not found. Out of this number, eleven cases were proved by inoculation to have had rabies. From these figures one may conclude that the finding of these lesions is practically conclusive evidence of the existence of rabies, and that in the failure to find them we have a possibility of error of about 5 per cent.

In investigating this subject at the Health Department laboratory, we have made use of material from the following sources, viz., 21 dogs, 24 guinea pigs, 10 rabbits, 3 horses, and 4 human cases. These figures represent the total number of cases of proved rabies as well as control cases. Of this number, there were 17 cases of rabies occurring naturally, *i. e.* from the bites of animals proved rabid. In 16 cases the disease was produced artificially in dogs, guinea pigs and rabbits, and 22 cases were used as controls. Of the 16 cases occurring naturally, 13 were dogs, 3 horses, and one a human case. All these cases showed the Negri bodies, the diagnosis being easily made by the microscopical method. By that I mean that after a search of about ten minutes at most of a single section of a small piece of either the cerebellum or ammons horn, or both, the diagnosis was made. In most of the cases a much shorter length of time was sufficient. In one of the experimental dogs the disease was produced by the inoculation of street virus in the region of a peripheral nerve, so that the condition would simulate that occurring naturally. This animal was allowed to go nearly to the time of death before being killed. The Negri bodies were found to be very numerous in the brain. Another

dog similarly inoculated was killed on the day he showed the first symptoms of nervousness. A careful examination of a section of both cerebellum and cerebrum failed to show the lesions, though it is possible that an examination of a number of sections might have revealed them. That the case was examined very early in the disease is shown by the fact that the submaxillary glands had not yet become infectious. By mistake, a portion of the brain tissue was not saved for inoculation. It may be said here that the majority of the dogs sent to the laboratory were killed during the course of the disease, some of them after only a day's sickness, yet the lesions were sufficiently pronounced to make the diagnosis easy.

In the rabbits and the guinea pigs the disease was produced by the subdural inoculation of the virus. All of these cases showed the lesions, but it is hardly fair to consider these cases as having equal importance with the others in estimating the diagnostic value of the method, inasmuch as it appears that there is a relation between the number of these bodies present in the brain and the proximity of the site of inoculation to the brain.

The control material was taken from the following sources: Five normal animals, three dogs suffering from unknown conditions, which were killed on suspicion of having rabies, but proven by animal inoculation not to have had the disease. In addition there were five cases of experimental diphtheria in guinea pigs, a case of staphylococcus infection including involvement of the brain in a rabbit, a case of general pneumococcus infection in man, one of traumatic cerebral hemorrhage in the human subject, and also one case of human tetanus and six cases of experimental tetanus in guinea pigs. In none of these cases was anything resembling Negri's corpuscles found, except in one of the cases of experimental tetanus. In this case there were a few minute eosinophile bodies occurring as inclusions in the Purkinje cells of the cerebellum. They showed no internal structure, and did not look exactly like even the structureless bodies found in rabies, and it is my feeling that one familiar with the appearance of these inclusions would not mistake them in making a diagnosis. However, it must be admitted that the similarity is sufficiently marked to be suggestive as to the nature of these cell inclusions. These diseases resemble each other in that in

both the virus travels by the central nervous system. We may suppose that all these inclusions—those showing the regular definite structure seen in rabies, together with the structureless forms seen both in rabies and occasionally in tetanus, are one and the same thing. In this case, of course, it must be assumed that they are all degeneration forms. Or it may be supposed that the structured forms found in rabies alone are the causative factor in this disease, while the other forms are the result of a poison acting especially on the nervous tissue and causing degeneration of its elements. So far as I know, the study of the morphology of these bodies has not progressed sufficiently far to throw light on this subject. However, as we are considering the diagnostic value of these lesions, it is to be noted, first, that even assuming their appearance identical in the two diseases, they are very commonly found in rabies, and are probably but rarely present in tetanus. Further, the dog, which is the animal most commonly afflicted with rabies, but rarely contracts tetanus. It would seem that much more extensive observation should be made on the histology of tetanus to clear up these points.

Even assuming that these lesions be definitely proven to be degenerations of the central nervous system, it seems quite possible that, when occurring in the numbers in which they do in rabies, they may still prove to be of great diagnostic value, since we must admit that, owing to the peculiar manner of transmission of the virus and the very long period of incubation and other peculiar features, this disease occupies a very unique position, and its pathology may be correspondingly unique.

With regard to the technique of the examination, one will find it necessary to take a small portion of the brain from both the cortex of the cerebellum and from the Ammon's horn, as the bodies are sometimes numerous in one region and not in the other. The tissue may be fixed in Zenker's fluid and stained with an eosin-methylene blue combination, in which case the inclusions stand out very clearly as red structures in the blue background of the cell body. Or absolute alcohol may be used as a fixative and hematoxylin and eosin as the stains. In this way a diagnosis may be made within twenty-four hours. The paraffin method of imbedding is to be preferred.

In conclusion, then, it would seem that in this method we have the means of making a rapid diagnosis, which is of about the same grade

of accuracy as that laboratory diagnosis of tuberculosis or diphtheria. That, further, the lesions are, as a rule, found early in the disease, and are not affected by changes in the brain tissue incident to the delay of shipping the material to the laboratory from a distance.

Further, the material for examination is easily obtained, and may be collected by any competent veterinarian and sent in the fixing reagent to the laboratory, thus saving time.

While further work on control diseases should be done to establish fully the standing of the method of Negri, it seems fair to conclude, from what has thus far been done, that we have in it a means of accomplishing what has long been sought, namely, a rapid diagnosis of rabies.

THE SMEAR METHOD AND FROZEN SECTIONS IN THE DIAGNOSIS OF RABIES.

BY IRA VAN GIESON, M. D.,

Assistant Bacteriologist.

During the past year experiments were made toward perfecting a method of rapid diagnosis of rabies in detecting the Negri bodies in smears of the central nervous system. It was thought that frozen sections of the brain might combine the advantages of the squeeze smear method with the natural topographical distribution of the Negri bodies which is somewhat disturbed in making the smear. After several trials, however, with variations of the Bevan Lewis method in which the sections of the congealed (but not frozen) brain tissue are immediately stained and dried on the slide, this procedure was abandoned. It was no comparison to the perfect demonstration with appropriate staining in the smear method, either for rapid diagnosis or detailed structural study of the Negri bodies. Frozen sections with similar technique of the salivary glands in various dogs revealed no traces of the Negri structures. Examinations also of the choroid plexus, pia mater, hypoglossal and motor oculi nerves, with methods similar to the smear procedure, yielded negative results as regards Negri structures.

The possibilities of the smear method in diagnosis in unfavorable material is shown in the instance of a dog received from Nanticoke, Pa., on February 8. The dog had been dead eight days; the dog's head was frozen stiff; the brain was solid, brittle and fragile. It was impossible to subject it to the smear manipulation. The brain could not even be removed without destroying all semblance to its topography. Accordingly the skull with partially exposed brain inside was placed on the radiator, and in three hours it had sufficiently thawed out into a partially grumous mass to permit of the smear procedure. In spite of these unfavorable conditions the first smear examined, which must have been from the neighborhood of the crucial fissure and motor zone—from the presence of the large Betz cells—showed the Negri bodies distinctly, although considerably deteriorated in the finer structural elements.

A few provisional experiments were made in connection with the smear method to determine the persistence of the Negri bodies. This is a matter of practical importance, since not infrequently animals suspected of rabies are sent to the laboratory for diagnosis a considerable length of time after death. Fortunately the morphologic diagnostic factor in rabies—the Negri bodies—show considerable resistance to post-mortem changes. Brains removed from street rabid dogs were kept in the ice box and examined at intervals for the Negri bodies by the smear method. The bodies, though much deteriorated in structure, could still be recognized 8 and 9 days after death, although at this period the brain had begun to undergo bacterial decomposition.

THE ROUTINE METHODS IN THE TREATMENT AND DIAGNOSIS OF HYDROPHOBIA USED IN THE DEPARTMENT OF HEALTH.

By D. W. POOR, M. D.

Assistant Bacteriologist.

Treatment—During the year 1905 there were treated 118 patients, an increase of 30 over the year 1904 and an increase of 50 over 1903. Of the 118 patients, 50 were treated at the laboratory, the other 68 having the treatment sent to them. Recently the treatment has been

changed slightly by intensifying it and lengthening the duration of it in severe cases to thirty days.

When the virus has been sent from the laboratory, each day's dose is mailed daily by special delivery. It is mixed with sterile glycerine and a somewhat longer and stronger treatment is given than would be the case if the patient were treated at the laboratory. The results have been as good in the patients treated away from the laboratory as in those treated here. Nevertheless it is recommended that severe cases be treated preferably at the laboratory, where this is possible. It is considered inadvisable to send the treatment in cases requiring more than two days for its delivery.

Diagnosis—The method of diagnosis by means of hardened sections of the brain, based on the discoveries of Negri, has been thoroughly tested, a portion of this work appearing in a reprint published from the Medical Record of April 15, 1905. This method had been found to be of considerable practical value, the time required for diagnosis being shortened to two days. This method of diagnosis has been materially improved upon by Dr. Williams, who has devised a method of examining smears made from the brain, the time required being shortened to an hour or even less.

Considerable work has been done in testing the therapeutic value of a protective serum in the treatment of hydrophobia. This work is not yet completed, but the results thus far obtained do not indicate the serum used to be of any value as an adjunct to the ordinary Pasteur treatment. In view of recent work with radium on the hydrophobia virus, experiments have been started in conjunction with Dr. C. B. Fitzpatrick along this line. A report of the work done to date follows:

A PRELIMINARY NOTE ON THE ACTION OF RADIUM UPON HYDROPHOBIA VIRUS.

BY DR. C. B. FITZPATRICK AND DR. D. W. POOR.

The encouraging reports of the investigations on the inhibitive effects of radium and its emanations upon the hydrophobia virus and upon the disease itself, as it occurs in experimental animals, has appeared to us to warrant further study of this subject.

Rehns¹ appears to have been the first to claim that the emanations of radium rendered the virus inert. Tizzoni² has made more extended studies of the effect of both the radium rays, and its emanations upon hydrophobia and its virus.

Tizzoni and Bongiovanni¹ state that the radiations of radium, rapidly decompose the fixed virus of rabies in vitro, and that when so exposed to these radiations, it loses its virulence after an exposure of two hours. They further state that the radiations have a similar effect upon the virus within a previously infected animal.

We have made two series of experiments with the virus in vitro, which appear to confirm to some extent these claims.

One-half of 1 c. c. of a fixed virus was placed in the bottom of a small tube. A small glass receptacle enclosing 100 milligrammes of the chloride of radium of 20,000 radio-activity of French manufacture, was suspended by means of a silk thread, so that the radium tube was placed directly within the center of the virus.

The virus was so treated for two hours.

Two guinea pigs were inoculated subdurally with this virus. Two controls being inoculated with fixed virus which had not been exposed to radium. The controls died one and two days earlier than the pigs which received the virus which had been exposed to the radium.

Both the untreated fixed virus and the radium virus were kept in the ice box over night before being inoculated.

TABLE I.

		Dec. 15.	Dec. 16.	Dec. 17.
Guinea pig I.....	Inj. Dec. 9 with Radium virus {	Somewhat weak. }	Dead.
Guinea pig II.....	Inj. Dec. 9 with Radium virus..	O. K.	Irritable.	Dead.
Guinea pig III.....	{ Control inoculated with fixed virus Dec. 9..... }	Dead.
Guinea pig IV.....	{ Control inoculated with fixed virus Dec. 9..... }	Dead.

A second experiment was made with 10 milligrammes of a German bromide of radium of about 1,800,000 radio-activity contained within an aluminum tube, sealed at both ends. This was enclosed in a cellu-

1. Compt. rendus hebdomadaires de la Societe de Biologie, 18 mars, 1905.

2. Acad. des Sciences de Bologne, au seance der 9 avril et dur 28 mai, 1905.

loid tube and placed in the middle of 1 c. c. of fixed virus, contained in a small test tube for two hours. This virus was then injected subdurally into guinea pigs and two controls were similarly injected with ordinary fixed virus. The controls died sooner than the ones inoculated with the radium virus, as indicated by the following table:

	Dec. 20.	Dec. 25.	Dec. 26.	Dec. 27.	Dec. 28.
Guinea pig I..	Inoculated with radium virus. }	Apparent-ly well. }	Sick.	Dead.
Guinea pig II.	Inoculated with radium virus. }	Apparent-ly well. }	Slightly weak. }	Sick.	Dead.
Controls	Inoculated with ordinary fixed virus..... }	Apparent-ly weak. }	Sick.	Dead.
Guinea pig III	Inoculated with ordinary fixed virus..... }	Slightly weak. }	Sick.	Dead.
Guinea pig IV					
Controls					

These two experiments seem to indicate that a two-hour exposure of a fixed virus (made from the cord in the dilution of about 1 part cord to 10 of salt solution), to the radiations of radium, renders it less active than the ordinary fixed virus of a similar dilution, which had not been exposed to radiations of radium.

Attempts were also made to treat animals sick with hydrophobia and which already showed some symptoms. Contrary to the reports of curative effects made by Tizzoni, we were not able to note any effect upon the course of the disease.

TABLE III.

		Dec. 21.	Dec. 22.	Dec. 23.
Rabbit I—Sick, 7 days after inoculation with fixed virus..... }	German radium of 1,800,000 radio-activity applied Dec. 20 to cornea of eyes alternately for one hour, then placed in position on cornea of right eye and left in place 18½ hours..... }	No change; weak.	Very weak.	Dead.
		Dec. 24.	Dec. 25.	Dec. 26.
Rabbit II—4 days after inoculation with fixed virus. }	Same specimen of radium applied December 22 to cornea of eye for 18½ hours..... }	No change; animal weak }	Very weak.	Dead.

A further experiment was made in treating a guinea pig with beginning symptoms due to street rabies by placing a small sealed glass tube containing 100 milligrammes of French radium chloride of 20,000 radio-activity under the skin and subcutaneous tissue along the spinal column. The animal treated in this way died one day sooner than the control, indicating that the radium had had no effect whatsoever.

One rabbit was also treated by exposing the dura mater at the site of the inoculation with a trephine. Immediately after the inoculation with a 1-100 dilution of fixed virus a flat-faced, disk-shaped receptacle containing the radium was applied over the opening. The side of the disk applied to the wound was enclosed by a thin mica plate and covered with gutta percha tissue. The radium was kept in place six days and then removed. The animal died two days later. 10 milligrammes of radium bromide of 10,000 radio-activity were employed.

Two sets of experiments were also made in which the virus was exposed for a longer period than two hours. These experiments showed a very slight inhibitive effect from 15 hours exposure and no effect from a 21-hour exposure. The fifteen-hour experiment consisted in using 100 milligrammes of French radium chloride and exposing about 9 c.c. of fixed virus (diluted to 1 to 10 solution), to its action. A very slight inhibitive action was noted in this experiment, as indicated in the following table:

TABLE IV.

	Dec. 9.	Dec. 14.	Dec. 15.	Dec. 16.
Guinea pig I.....	Radium virus inoculated.....	Apparently well.	Weak.	Dead.
Guinea pig II.....	" " "	"	Well.	Dead.
Guinea pig III.....	Fixed virus inoculated.....	Weak.	Dead.
Control				
Guinea pig IV.....	" " "	Apparently well.	Weak.	Dead.
Control				

The 21-hour exposure consisted in using the 11 milligrammes of German bromide of radium of 1,800,000 radio-activity and exposing 9 c. c. of fixed virus (diluted to a solution of 1 part of cord to 10 of salt solution) to its action for 21 hours. Practically no inhibitive action was observed in this experiment as is indicated by the following table:

TABLE V.

	Dec. 21.	Dec. 27.	Dec. 28.	Dec. 29.	Dec. 30.
Guinea pig I.....	Radium virus inoculated...	Dead.
Guinea pig II.....	" " " ...	Dead.
Guinea pig III } Control }	Fixed virus inoculated.....	Very weak.	Dead.
Guinea pig IV. } Control }	" " "	"	Very weak.	Very weak.	Dead.
Guinea pig V....	Radium virus inoculated. }	Apparent- ly weak. }	Paralyzed.	Dead.
Guinea pig VI....	" " " ...	Weak.	Completely paralyzed. }	Dead.

Guinea pigs I. and VI. in the above table were inoculated with a mixture consisting of equal parts of ordinary fixed virus, and the 21-hour radium virus used to inoculate number I. and II. with the idea of determining if the radium virus neutralized the fixed virus, the experiment appears to indicate that it has no effect upon the fixed virus.

Further experiments were made with weakened and diluted virus, which had been exposed to radium and controlled by similar weakened and diluted virus with the provoking result that all the controls as well as the treated animals have as yet, at the end of one month, shown no symptoms of the disease. A cord dried three days was used to inoculate in the first series of these experiments, after being exposed to radium as indicated in Table I.

In the second series of these last experiments 8 fine needle drops of a solution, made up of 1 part cord and 200 parts of salt solution, was placed within a capsule of gutta percha tissue and placed on the thin mica plate covering 10 milligrammes of pure radium bromide of 10,000 radio-activity. This solution was exposed in this way in the ice box for five days and then inoculated.

In the third series of these dilution experiments, two gelatine rods covered with a covering of a radium in such a manner that the alpha, the beta and the gamma rays all act, were surrounded in a tube with 2 c.c. of the 1-200 dilution of the fixed virus. This solution was also exposed for five days. These rods are called Lieber rods. The radium is kept in place by a covering which permits all the alpha rays to act.

The glass tube in the first experiment cut off all the alpha rays, and only permitted some of the beta and most, if not all, of the gamma

rays to act. The aluminum, the mica, gutta percha and celluloid coverings in the other experiments excluded all the alpha rays, a little of the beta and permitted all of the gamma rays to escape and act upon the virus.

The final series of the experiments with the diluted virus consisted of the injection of a mixture containing equal parts of 1.200 diluted virus, and of a fluid provisionally called X solution. This solution contained the emanations of radium, termed D. E. and F. and a minute quantity of the radium itself, in alcoholic solution. This solution also contained all the different rays and was of strong radio-activity. This solution mixed with the diluted virus was also inoculated into two guinea pigs, which have remained well for 28 days. Unfortunately, as already related the controls have also remained alive and well and we are consequently unable to judge what the results have been.

ON THE PRESENCE OF CERTAIN BODIES IN THE SKIN AND BLISTER FLUID FROM SCARLET FEVER AND MEASLES.*

BY CYRUS W. FIELD, M. D.

In December, 1903, Mallory¹ described certain protozoön-like bodies, which he had observed in the epithelial cells and in the lymph spaces of the skin, in material from autopsies on scarlet fever cases. He was unable to find them in the living patient.

At a meeting of the New York Pathological Society in April, 1904,² I reported that I had been able to find these bodies in the skin from five scarlet fever autopsies, but had been unable to find them in the skin taken from four living patients.

During the summer of 1904, Duval³ obtained bodies similar to those of Mallory in blister fluid from scarlet fever patients. In looking over

* Presented at the Fifth Annual Meeting of the American Association of Pathologists and Bacteriologists, Chicago, Ill., April 21, 1905.

1. Mallory—*Jour. of Med. Research*, 1904, x., 483.

2. Field—*Trans. of the New York Path. Soc.*, 1904, iv., 50.

3. Duval—*University of Pennsylvania Med. Bull.*, 1904, xvii., 298; *Virchow's Archiv*, 1905, clxxix., 485.

Duval's specimens I was struck by the close resemblance of many of them to the extracellular forms of the malarial parasite, except that in Duval's specimens these bodies showed no chromatin. His specimens were stained with Wright's modification of Leishmann's stain, which, in my experience, does not always give a chromatin reaction.

Since April, 1904, I have taken skin from twenty scarlet fever patients, ten scarlet fever autopsies, fourteen measles patients, four measles post-mortems, four patients with antitoxin rashes, and from five autopsies on diphtheria cases which had had a rash before death. Skin was taken from two children, one of whom had died of broncho-pneumonia, the other of marasmus. The skin from each of these cases was divided into four parts and placed in Petri's dishes. One was kept in the ice box, one at room temperature, one at 37° C., and one at 56° C. While able to obtain many kinds of degeneration products in sections made from these specimens (removed from the Petri's dishes every twenty-four hours under the four conditions), I did not find a picture similar to that shown in the sections from the material taken after death from the cases of scarlet fever and measles. Though many of the epithelial cells showed masses of varying sizes in their protoplasm, these inclusions showed a difference in staining reaction, some being more acidophilic than the surrounding cytoplasm, others less so, while some are basophilic to a marked extent.

The histological technique followed for all the material was fixation in Zenker's fluid, imbedding in paraffine, and staining, with eosin and methylene blue, the sections being, on an average, four microns in thickness.

The bodies found in the material from scarlet fever and measles were the same, so far as I could determine, as those described by Mallory. Some were intracellular, others lay in the lymph spaces. For the most part they were made up of a delicate reticulum which stained a light blue, the surrounding protoplasm being pink. Only a very few showed the rosettes Mallory described as being so characteristic. In the sections from measles the bodies were not so focal in location as those in scarlet fever and were found more often in the lymph spaces of the corium; there were also small bodies which showed no reticulum but did show a central nucleus-like granule, these small bodies being

found not only in the autopsy material, but also in that from the living patient.

So I can report that in sections made from the skin obtained after death from all of the fifteen cases of scarlet fever I have been able to find Mallory's bodies. One of these cases was most interesting in that two specimens of skin were obtained, one within five minutes after death and the other twenty-four hours later; in the section made from the former, no bodies could be made out, but in that from the latter these bodies were easily found. In the twenty-four cases of scarlet fever, where the skin was taken during life, no bodies were found, except in one section, where it was thought a single small body was seen, but as I have been unable to find it again, this cannot be considered a positive observation. In the material from the four autopsies on cases of measles, cellular inclusions were found in three, one being negative. In the last case the skin was taken one-half hour after death, and no other specimen could be obtained. In the material from the fourteen living patients no such bodies as Mallory describes were found, but all showed the small round nucleated bodies. The specimens of skin from the antitoxin rashes were negative, both from the living and the dead patient.

STUDY OF BLISTER FLUID.

Methods—The method used to obtain blister fluid was that devised by Duval except for a slight modification. A square of adhesive plaster two and one-half inches in size was covered with vaseline on its adhesive side, leaving a margin of one-half inch. A piece of blotting paper one-half inch in diameter and saturated with aqua ammoniæ fortior was placed in the center of this square, and the whole applied closely to the skin so as to admit no air. After being on from five to seven minutes, the skin was then exposed to the air, when in a short time a blister formed. The fluid was withdrawn from the blister with a sterile capillary tube. Moist spreads were made by blowing a drop of the fluid upon a clean slide and then placing on it a clean cover slip, under which the fluid was thinly and evenly spread. Smears were prepared and were fixed in absolute methyl alcohol for two minutes, some being fixed while dry, others while still moist. I did not find that it made any material difference as to which method was used.

In examining the smears many different stains were used, including a number of the various modifications of the Nocht-Romanowsky stain. In my experience Giemsa's⁴ stain was most satisfactory, and Hasting's⁵ was almost as good. Hasting's stain was slightly modified: instead of using 100 c. c. of methyl alcohol to dissolve the dye, I used 50 c. c. of glycerin heated to 60° C., and to this I added the dye, and then 50 c. c. of methyl alcohol which had been previously heated to 60° C. This idea was obtained from Giemsa's method. It permits of a greater concentration of the dye and the glycerin seems to prevent a deposit on the surface of the glass. In using these stains, it is well to over-stain and then decolorize in from fifty to seventy-five per cent. ethyl alcohol or in absolute methyl alcohol which gives a clearer picture.

Blister fluid was taken from eighteen cases of scarlet fever and from fourteen cases of measles. The bodies of Mallory were found in all the cases of measles and in fourteen out of the eighteen cases of scarlet fever.

Control Material—As control material blister fluid was taken from the following cases:

One case of erysipelas.

One case of eczema.

One case of erythema multiforme.

One case of urticaria.

One case of congenital syphilis.

One case of syphilis in the papular stage.

One case of irritated normal skin.

One normal individual.

One case of morbiliform antitoxin rash.

Seven cases of scarlatiniform antitoxin rash.

Material from pustules of two smallpox patients was also examined.

No bodies were found in the blister fluid from any of the above cases except in the last four of the scarlatiniform antitoxin rashes which were studied. In these four cases the blistering fluid was left on the skin for a longer period and caused a more severe irritation.

4. Giemsa, von—*Cent. für Bakt.*, 1902, xxxii., Abt. 1, 307, and 1904, xxxvii., Abt. 1, 308.

5. Hastings—*Bull. of the Johns Hopkins Hospital*, 1904, xv., 157; *Journal of Exper. Med.*, 1905, vii., 265.

In the material from these cases, bodies were found which it was impossible to differentiate from those found in the blister fluid of measles and scarlet fever. In one case after withdrawal of the material a moist spread was made and examined at once. Only a very few of the bodies were found, but a number of leucocytes were present. After six hours in the thermostat the preparation was examined again when many more of these bodies could be demonstrated. On making a smear and staining with Giemsa's solution, these bodies were indistinguishable from those in the blister fluid of cases of measles and scarlet fever. In these diseases, the bodies are found in the fluid as soon as the rash appears, but not before, and they can be found from four to six days after the appearance of the rash, but as soon as the rash fades away they disappear, becoming fewer and fewer until the sixth day, after which time not one has been observed. If two blisters are applied for from five to seven minutes to one patient, one blister being over a portion of the rash and the other on an area that has no eruption, the bodies can be found in the fluid over the rash; they are also present, though less numerous, in the fluid from the normal skin if the area is blistered for twice as long. The blister fluid from the rashes of both the measles and scarlet fever patients contained many more leucocytes than that from the other sources.

Conjunctival secretions from twelve cases of measles were examined. In the two cases where bodies similar in appearance to those in the blister fluid were found, there were numerous leucocytes, whereas in the ten negative cases the leucocytes were very few in number.

The Bodies—In smears of blister fluid stained with Giemsa's solution bodies of various kinds are found. The ones most commonly met with are those having a pale pink body with dark brown or black granules scattered throughout their substance. While many of these are undoubtedly red blood cells, or fragments of protoplasm of degenerating leucocytes, others are coagulated proteid, because similar structures can be found in smears made from horse serum which contained no cellular detritus. The bodies in which most interest centers are those which have the appearance of protozoa, many of them resembling closely the extracellular forms of the malarial parasite. These bodies have a pale blue protoplasm with one or more granules; the granules, which in

staining resemble chromatin, vary in size from a mere point to a particle taking up half of the total diameter of the body. Four times these bodies were found with the granules arranged about the periphery of the cell and with fine lines running to the center of the body, which gave them the appearance of a malarial rosette. The bodies ranged in size from one to fourteen microns in diameter, the majority being between three and seven microns. Those containing two or more granules were, as a rule, larger than those containing only one. In the moist spreads these bodies contained granules, dancing around in the protoplasm generally faster than the pigment of the malarial parasite. The morphology of these bodies in moist spreads and stained smears was therefore very strongly suggestive of protozoa.

The origin of these bodies, or bodies indistinguishable from them, was clearly made out. Leucocytes were very numerous in the moist spreads, particularly in those made with material from the acute exanthemata. When these spreads were watched in the warm box at 37° C., the pseudopodia of the leucocytes were seen to break off and in a short time assume a round form, each fragment containing one or more granules. When the pseudopodia which contained nuclear material had separated from the leucocytes and had assumed a regular outline, they resembled very closely individual cells. The reason the protoplasm of these bodies takes the weak basic dye instead of the acid dye is probably due to some chemical change that occurs when it separates from the cell. In some cases this protoplasm may be composed of nuclear material. In some of the stained smears leucocytes were found of which the protoplasm assumed this pale blue color, and in which the nucleus was undergoing karyorrhexis; this would indicate a degenerating cell. The nuclear fragments still gave the characteristic chromatin stain. Bodies of the same nature have been found when an emulsion of leucocytes in salt solution was left in the incubator for forty-eight hours, the salt solution having been previously diluted so as to make it hypotonic. The degenerating cells when stained gave some very beautiful pictures. (The differences in nuclear staining are shown in Plate XXVIII, Figs. 31, 32, 33, 34 and 38, *Journal of Experimental Medicine*, Vol. VII., No. 4, 1905.)

In this connection it may be of interest to note that Gotschlich,⁶ in a recent paper entitled "Ueber protozoen Befunde (Apiosoma) im Blute von Flecktyphus-Kranken," describes a parasite which he claims to be that of typhus fever. The "parasite" according to his description seems to be very similar to the bodies described by Duval in the blister fluid of scarlet fever and by myself in the same fluid from scarlet fever and measles. In an excellent study of the "Blood Changes in Typhus Fever," by Love,⁷ this author believes the parasite of Gotschlich to be nothing but degenerative changes in the red blood cells, and advances excellent arguments in favor of this hypothesis.

CONCLUSIONS.

I believe that the bodies found in sections of skin from cases of measles and scarlet fever are part of the protoplasm of the epithelial cells which has been so changed in its chemical nature that its staining reaction differs from that of the surrounding protoplasm. The small round extracellular bodies found in the living patients may arise from degenerating cells, but I cannot demonstrate this origin with certainty.

In sections of control and normal skin, the nuclei of the epithelial cells were often indented by the cell protoplasm, giving them an appearance similar to those indented by Mallory's bodies.

It would seem that if these bodies of Mallory's were protozoa they would have been found in the sections from both the living and the dead skin of scarlet fever and measles, as they were present in the blister fluid. Their absence is certainly more suggestive of a degeneration than of a protozoön. This view is also borne out by the fact that they were not found immediately after death, but were present in another specimen from the same case removed twenty-four hours later.

It would seem probable also that the bodies found in the blister fluid were the products of degeneration and cytolytic activity, because they were found in the antitoxin rashes as well as in the cases of scarlet fever and measles.

The histological changes in the skin of these two diseases leads us to expect the presence of cytolytic products both in the blister fluid and in the sections.

6. Gotschlich—*Deutsche med. Wochenschr.*, 1903, xxvi., 329.

7. Love—*Jour. of Path. and Bact.*, 1905, x., 296.

It certainly cannot be stated that none of these bodies is a protozoön, but it can be positively stated that a great majority of them arise from degenerating cells; and in many cases, I think, it is not possible to differentiate a degeneration from a protozoön by the study of its morphology and staining reactions.

The bodies present in blister fluid resemble very closely those granular bodies found in blood under certain conditions, and seen in vaccine lymph and in emulsions of tissues and in exudates. I think, therefore, that they are for the most part, if not wholly, products of degenerating tissue cells and of leucocytes, and within certain limits specific to scarlet fever and measles.

THE CONCENTRATION OF ANTITOXIN FOR THERAPEUTIC USE.

BY ROBERT B. GIBSON, PH. D.,
Bacteriologist.

The Department of Health of New York City since July 1st has been using extensively, and lately almost exclusively, an antitoxic fluid for diphtheria prepared by a concentration and purification of antitoxic serum. Accordingly some statement as to the nature of the product is desirable. A description of the process may serve the additional purpose of drawing attention to a subject which has of late been somewhat in the background. Both difficulties in the technique of handling the blood proteids and the general confusion of our knowledge of the serum globulins have discouraged to some extent the practical application of proteid chemistry to the concentration of diphtheria and tetanus antitoxins. That an artificial concentration is practicable has been experimentally demonstrated in this laboratory, where such a process has become a part of the routine work in the production of antitoxins.¹

The serum proteids precipitable by saturation with magnesium sulphate or by half-volume saturation with ammonium sulphate are of three general types—the fibrinogen, the eu- or more typical globulin, and the water-soluble or pseudoglobulin. Some confusion exists as to whether the term "euglobulin" is to be considered as that portion of

¹ From July 1st to December 31st about 60 liters of concentrated serum were prepared in the Research Laboratory and distributed for the use of the Department.

the serum proteids (exclusive of the fibrin precursors) precipitated by saturation with sodium chloride alone, or to that thrown out of solution by 36 per cent. volume saturation with ammonium sulphate—a concentration of the salt sufficing, according to Pick,¹ to differentiate chemically certain of the antitoxins and other immune substances associated with or actually comprising the serumglobulins. All the evidence at hand is in favor of the serumglobulin nature of antitoxins and some of the related bodies. The antitoxin of diphtheria developed by the immunization of the horse has every character of the soluble globulin in the serum from this animal. A concentration and purification of the antitoxic substance, at least until proteid chemistry is much farther advanced, must, therefore, be based on the separation of this soluble globulin.

The more recent attempts to characterize and differentiate individual serumglobulins have as a basis the association of various immune substances with the proteid precipitates obtained under certain conditions. Thus Belfanti and Carbone² found that diphtheria antitoxin was carried down in the globulins obtained by salting out with ammonium and magnesium sulphates, but not with the precipitates obtained by acetic acid. Dieudonné³ had previously shown that the proteids thrown out of solution by acetic and carbonic acids contained none of the antitoxin. Seng⁴ found that diphtheria antitoxin is precipitated along with the soluble globulins. Atkinson⁵ in this laboratory saturated with sodium chloride a solution of the moist serumglobulin precipitate obtained with magnesium sulphate, and by then employing heat differentiated the proteid into several fractions, all of which contained antitoxin; the protective properties corresponded quantitatively to the serumglobulin precipitates. Alteration of the proteid in the fractions by the addition of more of the sulphate produced proportionate changes in the distribution of the antitoxin. Brodie⁶ had previously carried out experiments somewhat similar to Atkinson's with similar results. Pick,⁷ on the con-

¹ E. P. Pick, *Beiträge z. Chem. Physiol. u. Path.*, i, p. 351, 1901.

² Belfanti and Carbone, *Centralbl. f. Bakteriol. (Ref.)*, xxiii, p. 906, 1898.

³ Dieudonné, "Ergebnisse der Sammelforschung über das Diphtherie Heilserum," *Arbeiten aus dem Kaiserlichen Gesundheitsamt*, xiii, p. 293, 1897.

⁴ Seng, *Zeitschr. f. Hyg.*, xxxi, p. 513, 1899.

⁵ Atkinson, *Journ. of Exper. Med.*, v, p. 67, 1901, and some unpublished experiments; see also Park, *Archives of Pediatrics*, Nov., 1900.

⁶ Brodie, *Journ. of Path. and Bact.*, iv, p. 460, 1897.

⁷ Pick, *loc. cit.*

trary, divided the serumglobulin into two parts by ammonium sulphate fractioning; with the one or the other of these fractions individual immune bodies were always associated. Pick ascertained that the fraction of the horse serum containing no antitoxin was precipitated by 36 per cent. volume saturation of ammonium sulphate solution; the protective portion then came down on the further addition of the precipitant to 44 per cent. Spiro¹ found that the difficultly-soluble globulin (obtained by dialysis) was precipitated by half saturation with potassium acetate. Using the method of Pick, Spiro associated the antirennin of horse serum with the euglobulin, which he considers identical with the half-saturation potassium acetate precipitate. Freund and Joachim² examined yet more closely the precipitation characters of Pick's fractions, finding for both the eu- and the pseudo- globulins soluble and insoluble parts. By the study of the precipitation limits of a number of immune substances, Porges and Spiro³ (without giving any of their experimental work) divide the serumglobulins into three distinct fractions, whose ammonium sulphate precipitation boundaries overlap unless the serum is greatly diluted. As the result of recent criticism, however, the differentiation of several soluble globulins is none too firmly established.⁴

The constant occurrence of the immune substances with the serumglobulins has suggested that these are actually a part of the one or the other of the globulin fractions. An increase in the globulin content of the blood as the result of immunization (Atkinson⁵ and others) is indicative of the serumglobulin nature of these bodies. Joachim,⁶ however, considered he found, in a single observation, that the increase was manifested in the non-protective fraction. Glässner,⁷ in a very recent paper, also states that immunization can be accomplished without any essential globulin change.

¹ Fuld and Spiro, *Zeitschr. f. physiol. Chem.*, xxxi, p. 132, 1900.

² Freund and Joachim, *ibid.*, xxxvi, p. 407, 1902.

³ Porges and Spiro, *Beiträge z. chem. Physiol. u. Path.*, iii, p. 277, 1903.

⁴ The purity of such ammonium sulphate fractions has recently been questioned by Haslam (*Journ. of Physiol.*, xxxii, p. 267, 1905); Osborne and Harris (*Am. Journ. of Physiol.*, xiii, 1905) have emphasized the untrustworthiness of this salt for theoretically differentiating proteids according to their precipitation limits.

⁵ Atkinson, *Journ. of Exper. Med.*, v, p. 47, 1901.

⁶ Joachim, *Arch. f. d. ges. Physiol.*, xciii, p. 558, 1903.

⁷ Glässner, *Zeitschr. f. exp. Path.*, ii, No. 1, 1905.

Attempts¹ to isolate and establish the non-proteid nature of diphtheria antitoxin and the other immune substances from the standpoint of their digestibility by trypsin have not given satisfactory results. These bodies appear to be very slowly attacked by this enzyme—a character that is possessed in a like degree by serumglobulins and is independent of the occurrence of an antitrypsin.²

In addition to the globulin separations of the type already discussed,³ methods especially directed towards isolating diphtheria antitoxin have been suggested. These include precipitation along with metallic hydroxides,⁴ combined sodium and potassium chloride separations,⁵ throwing down the antitoxin with zinc salts,⁶ and lastly by precipitating out the non-antitoxic proteids with potassium alum, and subsequently separating the globulins remaining in solution.⁷

The methods which have been proposed for the isolation or concentration of antitoxins, then, are for the most part peculiar and tedious ways by which the “globulins” were finally separated from serum and milk. Evaporation and freezing⁸ have been employed for concentration, but the use of these methods has not been continued. Pick states that by the isolation of his pseudoglobulin or higher ammonium sulphate fraction it is possible to concentrate the protective properties in a diluted serum ten to fifteen times. Pick's method is superficially the most practicable. Considerable quantities of antitoxin, however, may be carried down with the non-protective fraction⁹ on third saturation with ammonium sulphate solution (Brieger).

There is little record of the actual experimental administration of purified antitoxic globulins. Park¹⁰ studied the possibility of eliminating serum rashes by treating a considerable number of cases with an anti-

¹ Belfanti and Carbone, *loc. cit.*; Pick, *loc. cit.*; Brieger, *Festschrift für R. Koch*, Jena, 1903.

² Oppenheimer, *Beiträge z. chem. Physiol. u. Path.*, iv, p. 279, 1903.

³ Brieger and Ehrlich, *Zeitschr. f. Hyg.*, xiii, p. 336, 1893; Wassermann, *ibid.*, xviii, p. 236, 1894.

⁴ Aronson, *Berl. klin. Wochenschr.*, 1894, p. 425.

⁵ Brieger and Boer, *Zeitschr. f. Hyg.*, xxi, p. 259, 1896; Astros and Rietsch, *Compt. Rend. Soc. Biol.*, lii, p. 337, 1900.

⁶ Brieger and Boer, *loc. cit.*

⁷ Freund and Sternberg, *Zeitschr. f. Hyg.*, xxxi, p. 429, 1899.

⁸ Bujwid, *Centralbl. f. Bakt.*, xxii, p. 287; Ernst, Coolidge, and Cook, *Journ. Boston Med. Soc.*, ii, p. 166, 1898.

⁹ Some experimental observations on this subject showed me that antitoxin in a relatively large amount may be carried down with the lower fraction on third saturation with the sulphate. If the serum has been diluted several times, the precipitation results in a less noticeable loss.

¹⁰ Park, *loc. cit.*

toxic globulin prepared by Atkinson. Rashes were still produced. The therapeutic effects were no better than were obtained with ordinary serum, and the use of the separated product gave on the average no better results than the whole serum.

It is important at this point to emphasize the difference between *concentration* and the *practical concentration* of antitoxins. In the latter case the antitoxic globulins must be so prepared as to be ready for immediate administration; the sterility of the solution must be absolutely insured; the product must preserve its clarity better than ordinary serum, and when administered should cause no more irritation locally. If rashes can be altogether eliminated, or at least lessened, so much the better. Further, the keeping qualities must be unimpaired or improved. The method of concentration must be comparatively simple, certain and inexpensive.

Narrowed down by the conception of the proteid character of antitoxins, an artificial concentration for the present must consist in a separation of the antitoxic globulins. A concentration of more than three or four times is hardly practicable, for there is a limit to the amount of proteid which can be dissolved and to the viscosity of the fluid which can be sterilized through a Berkefeld filter. Failing to find Pick's fractioning entirely satisfactory, I precipitated the serum with an equal volume of saturated ammonium sulphate solution, filtered and extracted the residue with a saturated solution of sodium chloride. The antitoxic globulin is easily dissolved in the chloride solution in spite of the ammonium sulphate present, the non-soluble proteids (globulins, nucleo-proteids, etc.) sedimenting on standing. After filtering, the sodium chloride solution of the antitoxic globulins is precipitated by the addition of a half volume of saturated ammonium sulphate solution, or, better still, with acetic acid in the usual manner. The filtered precipitate is pressed dry with paper and dialyzed in parchment. If the acid precipitation has been employed, the globulin solution is neutralized in the course of the first few hours of dialysis, which is continued for from two to three days. Sterilization is accomplished by a double filtration through Berkefeld filters, one-half per cent. of sodium chloride being added and a preservative used. The potency of the product is ascertained, it is tested bacteriologically, and is finally injected into animals

and actually administered at the Department of Health hospitals before distributing.

The sodium chloride separation here suggested is to be preferred, in my opinion, to a simple precipitation or fractioning with ammonium sulphate. With the additional acid precipitation, almost all the sulphate is removed before dialysis. The resulting dilution is about the same as when the sulphate is employed. The antitoxin is practically all recovered and a concentration of between two and three times the original potency is easily and constantly obtained. The sodium chloride separation is a sharp one, the two groups of proteids showing essentially different physical characters as precipitates. The final product is no more viscous than ordinary serum; it is almost colorless, or tinged with hæmoglobin. When dried down at low temperature a beautifully transparent and entirely soluble scale antitoxin can be obtained. Large quantities of serum can be worked over at comparatively small expense.

Tests show that the artificially concentrated antitoxin, kept in small vials in an ice box in the usual way, preserves its potency as well as the ordinary antitoxic serum. Therapeutically the results obtained are practically identical with the beneficent effects commonly observed. Local irritation is no more marked and rashes seem to be less frequent and severe when the refined antitoxin is administered. Hundreds of cases have been treated with this product in the Department hospitals, yet no infection for which the antitoxin is responsible has resulted.

The method of separation is possible and practicable largely because of the extreme solubility of the antitoxic globulins and the remarkable retention of this character when compared with the behavior of other proteids under the same conditions. When precipitated with ammonium sulphate of only a fair degree of purity and when treated with saturated commercial sodium chloride solution, the moist precipitate retains its solubility for weeks. The soluble globulin can be repeatedly precipitated and purified to a high degree. The antitoxic properties follow this soluble globulin at every step and are lost or lessened only by such agents as can considerably modify the character of the proteid.

The highly purified soluble globulin, when practically salt-free, is in part precipitated by the addition of distilled water, when the reaction is appropriate; a trace of sodium chloride present brings the proteid

again into solution. Of practical importance, perhaps, is the fact that it is precipitated by the addition of an equal volume of alcohol—a resulting concentration in which sodium chloride is easily soluble. These and other chemical characters of the soluble globulins will be more fully discussed in a subsequent paper.

We are at present engaged in a study of the cause of serum rashes, etc., with preparations of the antitoxic globulins purified to a high degree. Some results already obtained with fairly pure experimental products indicate that the separation of the insoluble globulin does not by any means suffice to eliminate these deleterious effects. An 800-unit antitoxin preparation, fractionated and reprecipitated with ammonium sulphate solution, and subsequently extracted, first with acidified, and then with neutral saturated sodium chloride, dialyzed, and made slightly alkaline, produced a considerable number of severe rashes. It seems possible that the rash production in this instance was associated with some irritation due perhaps to a slightly excessive degree of alkalinity. I have also observed development of rash following the administration of an unneutralized acetic acid preparation of the antitoxic globulin. From the extensive use of ten of the latest routine preparations regarding which clinical reports have been received a very few mild urticarias only have resulted; in all of these preparations, the reaction (to litmus) has been neutral or but very faintly alkaline. The decrease in the frequency and severity of these effects reported from the Department hospitals has been very encouraging.

A more detailed technical description of the method of concentration which I have introduced into this laboratory follows. It is perhaps unnecessary to emphasize the care which should be exercised in working with any proteid solution which is to be injected for therapeutic purposes. Precautions which are of course second nature to the proteid chemist, may be neglected by assistants or others unfamiliar with this line of work. Serious consequences may follow any mistake.

For concentration, antitoxic serum of almost any grade or quality is serviceable; probably citrate, or other plasma could be used with success. Material of a low grade of protective power (150 to 250 units), old and returned stock, as well as highly potent serum, are all utilizable.

As deterioration is probably the result of autolytic processes,¹ just as concentrated preparations can be obtained from the old and returned stock as from the fresh serum.

Ten to fifteen liters of serum are precipitated by the gradual addition with stirring of an equal volume of saturated ammonium sulphate (Merck's pure crystalline, at ten cents per pound). After standing an hour or two the precipitate is collected on large folded papers on ribbed funnels. The precipitates are again dissolved in ten to twelve liters of water; the resulting solution is reprecipitated with ammonium sulphate solution in a volume equal to that of the water added. The precipitated globulins are once more collected on filters and then treated with twice the original serum volume of saturated sodium chloride solution. The sodium chloride extract is allowed to settle over night, and the supernatant solution of the antitoxic globulins is siphoned off and filtered. The insoluble residues are again extracted with salt solution, and the washings are combined with the first sodium chloride extract of the succeeding antitoxin preparation.

The sodium chloride extract is completely precipitated now either by the addition of about half its volume of saturated ammonium sulphate solution, or better, by the addition of about 0.25 per cent. of acetic acid. The final precipitate is filtered off. When sufficiently drained, the proteid and the containing papers are dumped on mats of filter paper so that the folded filters are extended into a semi-circle, while still holding the somewhat moist, soft, globulin precipitate. These precipitates are pressed out simply by occasionally changing the absorbent paper wrapped about them. When freed mechanically from the adherent folded hardened filters, the precipitate is placed in a bag of heavy parchment paper and dialyzed over night in running water; it is then neutralized if the acid precipitation has been employed. Dialysis is continued in running water for from two to three days or longer, toluol or chloroform being added as a preservative.

After filtering the dialyzed solution through paper pulp, it is roughly sterilized through a Berkefeld after about one-half per cent. of sodium chloride (c. p.) has been added. The antitoxin globulin solution is

¹ Atkinson noted a decrease in the magnesium sulphate precipitable globulins of old serum, the potency of which had deteriorated correspondingly.

again sterilized by a filtration through a second Berkefeld. A preservative is finally used. In filtering the first few cubic centimeters should be discarded or turned into the next preparation.

The protocols of two of the earlier experimental preparations and of a subsequent concentration are given below. No attempt was made to make the chemical technique a very careful procedure—*i. e.*, the insoluble globulin precipitates were not thoroughly extracted, etc. As it is, they show at least about four-fifths of the antitoxic property recovered:

Preparation XV. 9000 c.c. serum; potency 200, net total anti-toxin (corrected for two large samples taken during preparation)	1,672,000 units.
Final product (500 units per c.c.)	1,400,000 "
Preparation XVI. 9000 c.c. serum; potency 300; total	2,700,000 "
When neutralized after 18 hrs. dialysis 2620 c.c. (900 units)	2,358,000 "
Final product, 3320 c.c. 700 units per c.c.	2,324,000 "
Preparation AIV. 60 liters serum, potency about 275, were concentrated to a final volume of 19 liters, potency 800 units per c.c.	

It is frequently stated¹ that artificial concentration of antitoxin is superfluous, because it is possible to immunize animals to such a high degree that any further procedure is unnecessary. The production of a highly potent serum in any horse, however, is an uncertain process. For this purpose only 25 per cent. of the horses tried by us are serviceable, and even if suitable, the horses can be used for only a few months when the immunization is forced to produce a grade of 500 units per cubic centimeter. An artificial concentration of low-grade serum is possible probably at no more, and perhaps even less, expense than obtains in the present procedure for producing an equally potent antitoxin; considerable saving may also result from utilizing serum of a grade below 200 units, as well as the returned serum, which is at present usually destroyed. A further reduction of expense by working over citrate-plasma instead of serum is possible. Again there may arise occasions in any serum laboratory when the production of a supply of highly potent antitoxin at short notice is most desirable. For circumstances of this character an available method for artificially concentrating the protective properties of serum might be of considerable service.

¹Oppenheimer. *Toxins u. Antitoxine*, 1904, p. 87.

The elimination of the serum rashes, even in part only, makes the expense question almost a negligible factor in the concentration and refining of antitoxins.

The work so far done and planned is somewhat comprehensive in scope and is both practically and scientifically important. It has to do not solely with the concentration of antisera and the elimination of the serum rashes, etc., but may also throw some light on the chemical characteristics and the nature of antitoxins and related substances, and on the probable functions of the so-called "serumglobulins."

In concluding, I desire to express my thanks to Dr. William H. Park, director of this laboratory, for his active and helpful co-operation.

SOME NOTES ON THE CONCENTRATION OF DIPHTHERIA TOXIN.

BY EDWIN J. BANZHAF,

Assistant Chemist, Research Laboratory.

Last summer we experienced some difficulty in producing a diphtheria toxin of such a strength that one cubic centimeter contained from 200 to 300 minimal lethal doses for 250 gram guinea pig in four or five days, which has been the usual strength used by us in injecting the horses.

Toxic fluid of half this strength necessitates injections of twice the amount and over. This, in cases of horses highly immunized, amounts to a liter or more for each injection per horse, causing at the seat of injection marked inflammation and swelling, which sometimes results in the formation of abscesses. After a few months of repeated large injections, the tissues become indurated, and the horses suffer in general health. Injected into a horse, the same number of guinea pig fatal doses, when in a concentrated solution, yield a higher grade of antitoxin in a given time than the same number of guinea pig fatal doses in a dilute solution.

In consequence of this, the study of concentration of the diphtheria toxin was taken up.

Since December, 1905, by a process of concentration (alcohol precipitation), I have eliminated a large part of the non-toxic constituents

of the diphtheria toxic broth ; thus concentrating the toxin 10 to 20 times. This is shown by the following examples :

From 12 liters of diphtheria toxin, strength 25 M.L.D. per c.c., obtained a product of 542 c.c. strength 500 M.L.D. per c.c. A loss of about 10 per cent. toxin.

From 10 liters of diphtheria toxin, strength 50 M.L.D. per c.c., obtained a product of 453 c.c. strength 1000 M.L.D. per c.c. A loss of about 10 per cent. toxin.

From 8 liters diphtheria toxin, strength 100 M.L.D. per c.c., obtained a product of 600 c.c. strength 1200 M.L.D. per c.c. A loss of 10 per cent toxin.

We have immunized four new horses at the antitoxin station with a concentrated and refined toxin, containing 500 M. L. D. per c.c. The results of these are as follows :

Two of the horses responded remarkably well, yielding, the one, a serum containing over 1,400 units per c.c. and the other, a serum of over 1,300 units. The other two horses produced so little antitoxin that they were not continued in the service.

The first named horses have continued producing strong antitoxin and have kept in remarkable physical condition despite the oft-repeated bleedings. It is probable that this is largely due to an elimination of deleterious substances in the toxic bouillon.

REPORT ON THE DIPHTHERIA ANTITOXIN HORSES.

BY EDWIN J. BANZHAF,

Assistant Chemist Research Laboratory.

The total production of diphtheria antitoxic serum for 1905 amounted to 442,755 cubic centimeters. This amount was produced from 20 horses. Seven of these horses had only one bleeding, and of these seven, four died soon after their initial bleeding. The other three were new horses, having entered the antitoxin station in August, 1905, and being bled for the first time in December.

Horses 239, 244 and 246, which entered the antitoxin station October 12, 1903, January 15, 1904, and January 19, 1904, respectively, produced during the spring and summer of 1904, a high grade antitoxic serum,

the quantity of which, however, diminished very rapidly toward the close of the year. After the second bleeding of this year, horses 239 and 244 died, and after the fourth bleeding, horse 246 died.

Horses 269 and 270, which entered the antitoxin station May 24, 1904, died after the fourth bleeding of this year.

Horse 234, the oldest diphtheria antitoxin horse at present in use, entered the antitoxin station August 18, 1903, and has produced a fairly high grade antitoxic serum for a period of 26 months. The two last bleedings of this horse December 26, 1905, and December 29, 1905, gave 550 and 450 units per cubic centimeter, respectively.

Horses 262 and 264, which entered the antitoxic station on February 12, 1904, and February 26, 1904, have produced high grades antitoxic serum for a period of 18 months. Their two last bleedings, December 26, 1905, and December 29, 1905, gave 550 and 450 units per cubic centimeter, respectively.

The bleedings from January to July of this year averaged 400 units per cubic centimeter. From July to December of this year considerably less.

The large amount of antitoxic serum of 300 units and under, was due to frequent bleeding of the horses after July of this year.

This low grade serum was utilized by concentration as is discussed elsewhere in this report. (See Dr. Gibson's article.)

The following table gives the production and strength of the diphtheria antitoxic serum obtained from the horses during the year.

Entered Antitoxic Station.	Number of Horse.	Number of Bleedings.	Total Diphtheria Antitoxic Serum. C. C.	500 Units or Over per C. C.	400 to 500 Units per C. C.	300 to 400 Units per C. C.	Below 300 Units per C. C.	Remarks.
August 18, 1903.....	234	11	60,540	6,540	20,970	27,880	5,150	Still in use.
October 12, 1903.....	239	2	7,240	4,080	3,160	Died May 12, 1905.
January 14, 1904.....	244	2	10,100	4,940	5,160	Died April 17, 1905.
January 19, 1904.....	246	4	21,000	15,030	5,970	Died May 12, 1905.
February 12, 1904.....	262	13	70,085	11,160	11,510	24,140	23,275	Still in use.
February 26, 1904.....	264	11	40,740	8,040	10,430	14,930	7,340	Still in use.
April 16, 1904.....	268	11	36,380	12,920	14,000	6,730	2,730	Still in use.
May 24, 1904.....	269	4	17,560	4,850	7,820	4,890	Died May 15, 1905.
May 24, 1904.....	270	4	19,120	5,960	5,350	3,790	4,020	Died May 12, 1905.
October 31, 1904.....	271	9	43,110	6,530	24,280	12,300	Still in use.
October 31, 1904.....	272	8	36,215	3,950	8,930	4,610	18,725	Still in use.
December 22, 1904.....	273	1	3,390	3,390	Died July 31, 1905.
May 11, 1905.....	280	1	7,640	7,640	Died Sept. 18, 1905.
May 22, 1905.....	282	1	4,370	4,370	Died Dec. 29, 1905.
July 6, 1905.....	284	5	14,190	7,390	6,800	Still in use.
July 28, 1905.....	285	5	24,535	5,460	5,540	13,535	Still in use.
August 12, 1905.....	286	1	5,010	5,010	Still in use.
August 14, 1905.....	287	1	12,540	12,540	Died Dec. 7, 1905.
August 21, 1905.....	288	1	5,250	5,250	Still in use.
August 21, 1905.....	289	1	3,740	3,740	Still in use.
	20	96	442,755	58,880	106,110	176,965	100,800	Died during the year. 9.

THE VALUE OF DIPHTHERIA ANTITOXIN IN THE TREATMENT OF DIPHTHERIA AS ESTABLISHED BY TEN YEARS OF TRIAL.

BY WILLIAM H. PARK, M. D., AND CHARLES BOLDUAN, M. D.

It seems wise before entering on a consideration of statistical and other reports to consider certain facts which should be understood in order to make such a study valuable.

The history of the disease now known as diphtheria goes back to the remotest times, descriptions of the malady being found in the writings of some of the old Greek writers. Even during recent years the disease in its different localizations in the body has been known under different names, and this must be recognized or very faulty conclusions may be drawn.

In 1765 Francis Horne, M. D., of Edinburgh published a pamphlet entitled, "An Inquiry into the Nature, Cause and Cure of Croup." The word "croup" at that time was the term given to diphtheria, the Scotch word "croup" meaning to croak or to speak with a harsh voice. Horne's account of the autopsy findings would describe the cases as met with to-day and is extremely interesting.

In 1771 Dr. Samuel Bard of New York wrote an elaborate article on the "Cause, Nature and Treatment of Suffocative Angina." His description of the disease which we at once recognize as diphtheria is extremely vivid, but his knowledge of the etiology is somewhat vague.

The present name was given to the disease by Bretonneau in 1821. His observations were so extensive and so correct that little advance in knowledge took place until the causal relations of the diphtheria bacilli and their associated microorganisms to the disease began to be recognized.

In the official death returns in New York we first find the term diphtheria used in 1857, when two deaths are reported as due to this disease.

The following table is of interest as showing the names under which formerly the disease was undoubtedly returned in New York, and how the use of the term diphtheria rapidly spread.

Year	Inflammation of Throat.	Inflammation of Tonsils.	Quinzy.	Sprue.	Ulceration of Throat.	Angina.	Croup.	Diphtheria.	Totals.
1851.....	53	78	5	38	462	636
1852.....	50	33	20	43	595	641
1853.....	107	43	5	16	502	673
1854.....	118	23	..	90	12	10	637	772
1855.....	64	13	..	66	10	12	639	804
1856.....	50	8	4	49	7	14	550	682
1857.....	71	15	10	36	7	21	560	2	622
1858.....	70	15	2	56	7	11	478	5	644
1859.....	111	4	3	24	19	58	622	53	894
1860.....	132	..	6	15	..	37	599	422	1,211
1861.....	101	25	17	16	460	453	1,072
1862.....	68	27	7	2	685	594	1,383
1863.....	49	14	25	64	908	981	2,041
1864.....	18	..	1	4	8	37	754	781	1,603
1865.....	4	24	5	449	534	1,016

So far as the change in nomenclature is concerned it may be well to quote the Registrar General's Report for England and Wales, 1898. The conditions described therein undoubtedly apply to a greater or less extent to all other cities and countries:

"With regard to the changes that have taken place from time to time in the nomenclature of diphtheria it is important to bear in mind the following points: 1. Diphtheria as a distinct affection had scarcely been recognized in England previous to 1855, in which year this disease was for the first time separated from scarlet fever in the national records of the causes of death. 2. The great diphtheria epidemic of 1858-9 was preceded by a marked increase in the mortality from croup and quinzy, by a still greater increase in the mortality from cynanche maligna, and also by some increase in that from laryngitis. 3. The diphtheria epidemic of 1863 was accompanied by parallel movements in the mortality ascribed to croup and also in that ascribed to

cynanche maligna. 4. The incidence of croup in regard both to season and to the ages of death of those attacked is found to have been similar to that of diphtheria. 5. Whereas the deaths attributed to croup in 1861-70 were more numerous by one-third part than those attributed to diphtheria, the deaths attributed to croup in 1896-98 were less than one-sixth part as many as the deaths attributed to diphtheria. These facts taken in conjunction afford statistical support to the current medical opinion as to the identity of the two diseases, and certainly warrant the assumption that by far the greater number of deaths hitherto attributed to croup have really been caused by laryngeal diphtheria. * * *

“At the present day the proportion of deaths attributed to ‘croup’ is relatively so small as to scarcely affect the rate. This is especially true for London, where the mortality from ‘croup’ barely exceeds 3 per cent. of that from diphtheria.”

This gradual change in nomenclature is well shown by a table giving the annual average rates of mortality for each of several groups of years in England and Wales (Registrar General’s Report 1898). The figures are per 1,000,000 inhabitants.

Periods.	Diphtheria.	Croup.	Diphtheria and Croup.	Laryngitis.	Sore Throat and Quinzy.
1861-1870.....	185	246	431	51	14
1871-1880.....	121	168	289	48	10
1881-1890.....	163	144	307	54	24
1891-1895.. ..	254	70	324	48	21
1896-1897.....	269	43	312	44	19
1898.... ..	244	27	271	38	17

This change is particularly well shown in the table given on page 40 (1851-1865) and by the following continuation also from the New York City records:

Year.	Croup.	Diphtheria.	Approximate Ratio.	Year.	Croup.	Diphtheria.	Approximate Ratio.
1858.....	478	5	96.0:1	1878.....	499	1,007
1859.....	622	53	12.0:1	1879.....	522	671
1860.....	599	422	1.4:1	1880.....	910	1,390
1861.....	460	453	1.0:1	1881.....	1,038	2,249
1862.....	685	594	1.1:1	1882.....	729	1,525
1863.....	908	981	.9:1	1883.....	644	1,009
1864.....	754	781	1884.....	748	1,090
1865.....	449	534	1885..	855	1,325
1866.....	368	435	1886.....	968	1,727
1867.....	338	251	1887.....	889	2,167
1868.....	342	276	1888.....	639	1,914
1869.....	483	328	1889.....	605	1,686
1870.....	421	308	1890.....	521	1,262
1871.....	466	238	1891.....	609	1,361	0.4:1
1872.....	675	446	1892.....	670	1,436	0.4:1
1873.....	732	1,151	0.6:1	1893.....	588	1,970	0.3:1
1874.....	594	1,665	0.5:1	1894.....	511	2,359	0.2:1
1875.....	758	2,329	0.3:1	1895.....	342	1,634	0.2:1
1876.....	527	1,750	0.3:1	1896.....	208	1,555	0.1:1
1877.....	472	951	0.5:1				

The following table illustrates a similar change in nomenclature in Boston. (John McCollom, Boston Medical and Surgical Journal, June, 1905.) Taking the yearly percentages of the deaths from diphtheria and croup to the total mortality we get the following:

Year.	Diphtheria.	Croup.	Year.	Diphtheria.	Croup.
1879.....	5.285	2.081	1885.....	3.472	1.299
1880.....	6.892	2.180	1886.....	3.519	1.014
1881.....	6.665	2.229	1887.....	3.137	.933
1882.....	5.091	1.300	1888.....	4.609	1.167
1883.....	4.568	1.673	1889.....	5.498	1.160
1884.....	3.585	1.475	1890.....	3.938	.599

Year.	Diphtheria.	Croup.	Year.	Diphtheria.	Croup.
1891.....	2.194	.501	1898.....	1.562	.138
1892.....	3.684	.596	1899.....	2.480	.242
1893.....	4.064	.597	1900.....	4.598
1894.....	7.092	.529	1901.....	3.124
1895.....	5.190	.582	1902.....	1.730	.319
1896.....	4.435	.481	1903.....
1897.....	3.684	.403			

In other words, whereas, the proportion of croup to diphtheria was formerly from 1:3 to 1:4 it has fallen to 1:9 to 1:10 or more.

THE INFLUENCE OF BACTERIOLOGICAL EXAMINATIONS ON DIAGNOSIS.

In 1883 Klebs demonstrated the existence of a bacillus in the false membrane of diphtheria. In the following year Löffler isolated and cultivated this organism. Since that time the term diphtheria has been limited to an exudate or pseudo-membranous inflammation characterized by the presence of the diphtheria bacilli.

With the increase in our knowledge we have learned that a very similar clinical and pathological picture can be produced by other bacteria, especially by streptococci. The more severe of these cases were formerly classed as diphtheria and require to be regarded in any statistical study. The less severe cases as well as the milder cases of true diphtheria were in some cases classed as diphtheria and sometimes as not according to the learning of the physician. It is disputed by many whether the bacteriological diagnosis adds to or diminishes the number of cases classed as diphtheria. It is certain that some very mild sore throats which would not clinically be classed as diphtheritic are now often classed as diphtheria; on the other hand, however, there are many cases presenting well marked membranes on the tonsils and other clinical symptoms of diphtheria and many exudates complicating scarlet fever which bacteriological diagnosis throws out as cases not diphtheria.

In the Annual Report of the Health Department of the City of Boston, for 1905, this question is discussed as follows:

“In previous reports (1902-1903) calculations have been given to show by two different methods the probable error of the physician in

the purely clinical diagnosis of diphtheria. These calculations indicated that at least 38 per cent. of the cases reported without bacteriological examination as possibly diphtheria, are really not diphtheria. In Boston, from 70 to 80 per cent. of the total reported cases receive bacteriological confirmation. It seems clear that by means of cultures in Boston less cases remain under the designation of diphtheria than if these cases were judged solely on clinical evidence."

Our own opinion is that there is probably a slight increase in the number of mild diphtheria cases reported because of the making of cultures. These cases even if suspected to be diphtheria by the attending physician would otherwise often go unreported.

THE VALUE OF HOSPITAL STATISTICS IN ESTIMATING THE RESULTS OF TREATMENT.

A difficulty in estimating the effect of antitoxin hospital practice is the fact that in recent years many cases are sent to hospitals in some cities which formerly did not reach there, cases sent to prevent contagion. Thus in Berlin in 1884-1887 only 26-30 per cent. of the total number of cases of diphtheria were sent to hospitals. In 1897 there were 57 per cent.; in 1898, 60 per cent. and in 1899, 63 per cent. so treated. (Gottstein, *Therapeut. Monatshefte*, 1901, p. 605.) On the other hand, in New York there has been no increase of hospital facilities and there has been no appreciable change in the percentage of cases treated in the hospitals.

THE MORTALITY IN CASES TREATED EARLY IN THE DISEASE AS CONTRASTED WITH THOSE TREATED AT A LATER PERIOD.

In view of the inconclusive results of comparisons of case mortalities before and since the introduction of antitoxin treatment, as to the exact amount of improvement, a number of observers have sought to show the value of serum treatment by tabulating the days on which such treatment was begun and the corresponding mortality. The antitoxin being unable to materially influence diphtheria in the majority of cases after the fourth day. Thus Faber (*Jahrb f. Kinderheilk*, 1904. No. 59) publishes his analysis of 3,137 cases of diphtheria occurring in

the Blegdams Hospital in Copenhagen. He excludes cases complicated with scarlet, whooping cough and other diseases unless these had already passed their height and were receding.

Commencement of Serum Treatment.	Number of Patients.	Number of Deaths.	Mortality.	Calculated Number of Deaths According to the Entire Mortality of the Growth (11.5 per cent.).	Difference Between Actual and Calculated Mortality.
1st day.....	99	7	7.1	11	—4
2d “	641	48	7.5	74	—26
3d “	763	69	9.0	88	—19
4th “	555	63	11.4	64	—1
5th “	334	52	15.6	38	+14
6th “	171	29	17.0	20	+9
7th “	80	17	21.3	9	+8
Later than 7th	196	39	19.9	23	+16
Unknown	298	35
Total.....	3,137	359	11.5

Average, 11.5 per cent.

Admission on the—	Mortality. Per Cent.
2d day of disease.....	19
3d “ “ “	24
4th “ “ “	38
5th “ “ “	30
6th “ “ “	21
7th “ “ “	41

Bing and v. Ellerman (Therap. Monatshefte, 1904, XVIII., p. 398) have critically studied a large number of cases with a view to refuting such an interpretation. They cite Heubner, who had caused the prognosis to be written opposite each history on the admission of the patient. The results showed that the patients admitted later were more apt to die mainly because they were admitted in worse condition than the others. Heubner's results may be tabulated as follows:

	Unfavorable Prognosis Per Cent. of Cases.
Admission on—	
1st day	6%
2d "	8%
3d "	14%
4th "	17%
5th "	22%
6th or 7th days.....	53%
8th and later.....	69%

Bing and v. Ellerman then give their own report on 1,356 cases of diphtheria from the preantitoxin days (1889 to 1894) occurring in the Blegdams Hospital in Kopenhagen.

	Number Admitted.	Of these there died.	Per Cent. Mortality.
Day of Admission—			
1.....	113	38	34%
2.....	494	110	22%
3.....	350	95	27%
4.....	177	68	38%
5.....	125	53	42%
6.....	54	22	41%
7.....	23	13	57%
8 and later.....	20	12	60%

They analyze their figures as follows:

“ If the mortality is calculated for periods of 48 hours instead of 24 hours the irregularities disappear and one obtains the series 24, 31, 42, 58 per cent. At first sight one would be inclined to agree with Glaeser and others, especially when one remembers that in the last 10 years the mortality of diphtheria has been decreased only from 12 to 4 per cent. and that this decrease includes with the effect of treatment and the spontaneous decrease in virulence which the disease has under-

gone. If our curve of the preantitoxin day is compared with that of Jellineck from the serum period or with Faber's given above, one will notice that the rise of the curve is more rapid in the serum period. Furthermore in the serum period one does not see the relatively high mortality on the first day admission which we found."

THE SEASONAL AND CLIMATIC OCCURRENCE OF DIPHTHERIA.

If we glance for a moment at the seasonal and climatic distribution of diphtheria, we find that although the disease has prevailed under all circumstances of climate, in the highest as well as in the lowest latitudes, along the coast as well as in the interior, it is particularly in the temperate and colder zones that it effects its greatest ravages. Even allowing for the defective data from equatorial and subtropical countries, we find that there the disease is quite rare.

Hirsch gives the following data regarding the incidents of season and weather which shows that even in temperate climates where diphtheria is prevalent in cold weather it is greatly lessened with the approach of summer.

	Period.	SEASON.			
		January to March.	April to June.	July to September.	October to December.
Sweden.....	1861-1870	31.0	20.9	19.0	29.1
Berlin.....	1876-1883	24.2	21.4	22.1	32.3
Saxony.....	1873-1878	28.7	17.6	17.7	36.0
Hamburg.....	1873-1882	23.9	24.2	21.7	30.2
Göttingen.	1878-1882	33.5	22.4	19.5	24.5
Schleswig-Holstein	1872-1881	29.6	20.0	22.0	28.4
St. Petersburg.....	1878-1882	24.7	23.1	20.9	32.3
Frankfurt	1863-1883	27.3	24.7	19.6	28.4
Vienna	1863-1883	31.3	23.5	15.7	29.5
Philadelphia.....	1868-1875	24.0	21.4	18.5	37.1

These three-month periods, while they do show the lowest distribution of diphtheria in the third, *i. e.*, summer period, do not reflect the influence of weather as well as the following chart arranged by weeks.

This shows the deaths per 100,000 in New York (old city) in 1903 to 1905, inclusive. Since it is impossible to accurately reflect the meteorological conditions we have plotted on this curve the deaths from pneumonia and bronchitis. These ought to be a fair measure of the effect of the weather in producing colds or other diseased conditions of the respiratory tract. Any cold wet weather with slush in the streets is almost certain to be followed by an increase in inflammations of the respiratory tract and in diphtheria.

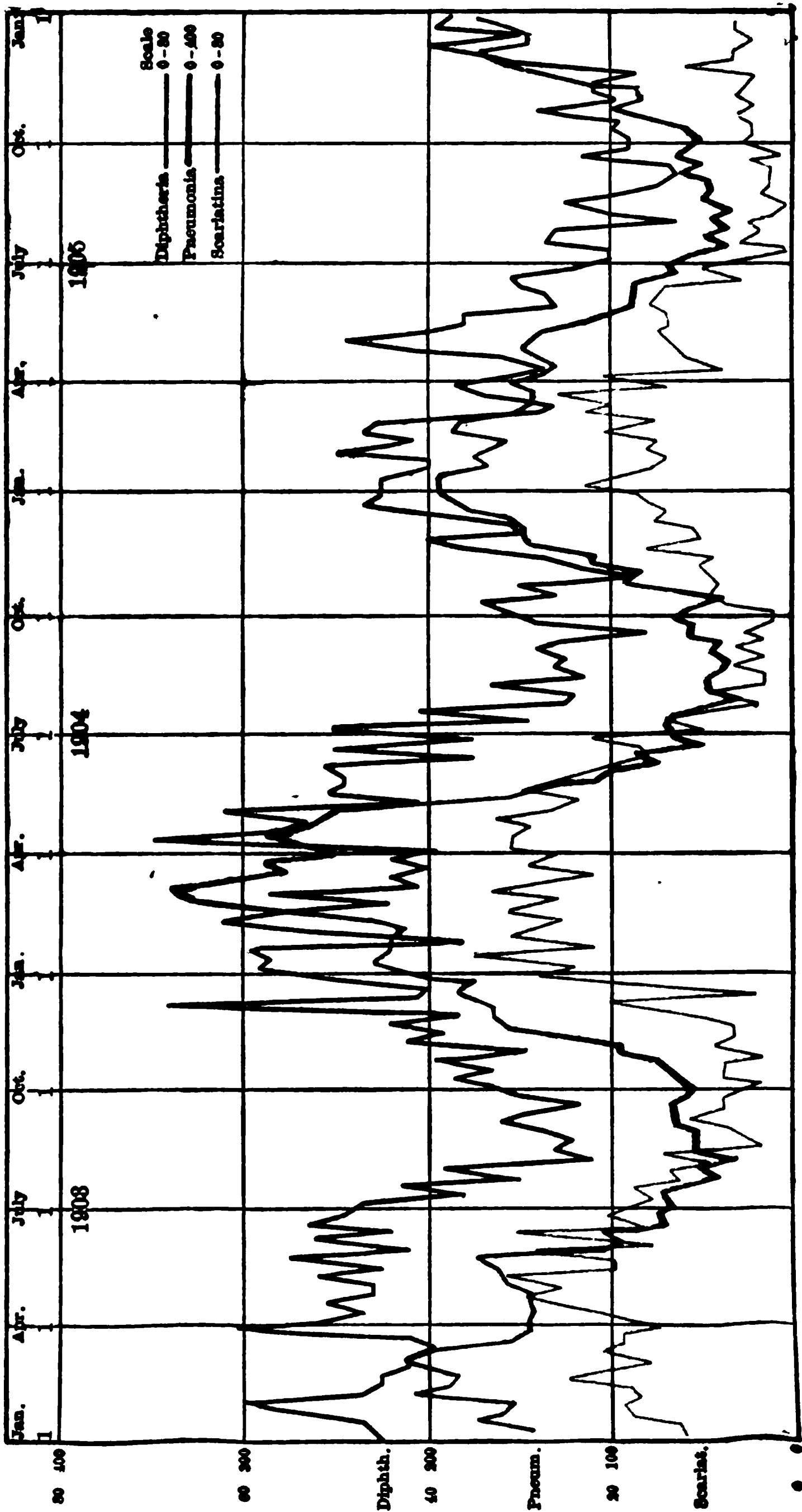


Fig. I.

Here we see that the disease is at its ebb point during the middle of summer and gradually increases in extent from about the beginning of October. In this *rise* it follows rather closely the curve of pneumonia and bronchitis—*i. e.*, so far as we can tell, the effect of the weather. Again taking the pneumonia-bronchitis curve as an index, we see that although the effects of severe weather become much less about the first week in May, the curve of diphtheria continues for about a month longer. It is evident that this is due to the fact that the diphtheria bacillus is able in many to excite diphtheria without any aid from atmospheric conditions while the pneumo-coccus as a rule requires such assistance.

In the case of scarlet fever, Dr. A. Seibert has published charts showing that the prevalence of the disease coincides very closely with the sessions of school. In many years, however, this influence is not so noticeable although always an important factor.

To what extent this holds for diphtheria can be seen from our chart, which also has plotted on it the curve for scarlet fever. While the study of individual cases proves that the spread of diphtheria is aided by school attendance, the curve for large cities does not follow the sessions of school very closely as the weather is an even more important factor.

CHARACTERISTICS OF EPIDEMICS.

Statistical studies in order to be of great value must embrace a sufficient period of years and a sufficiently large number of cases. This is particularly true of diphtheria, a disease which recurs in epidemic cycles of varying length. This is well shown by the following curves, perhaps the most accurate that can be found anywhere.

percentage
 from
 1855-1895

Fig. 2.

KOPENHAGEN: Cases and deaths from diphtheria, 1855-1895: their variation from the mean of the entire period. (After Newsholme.)

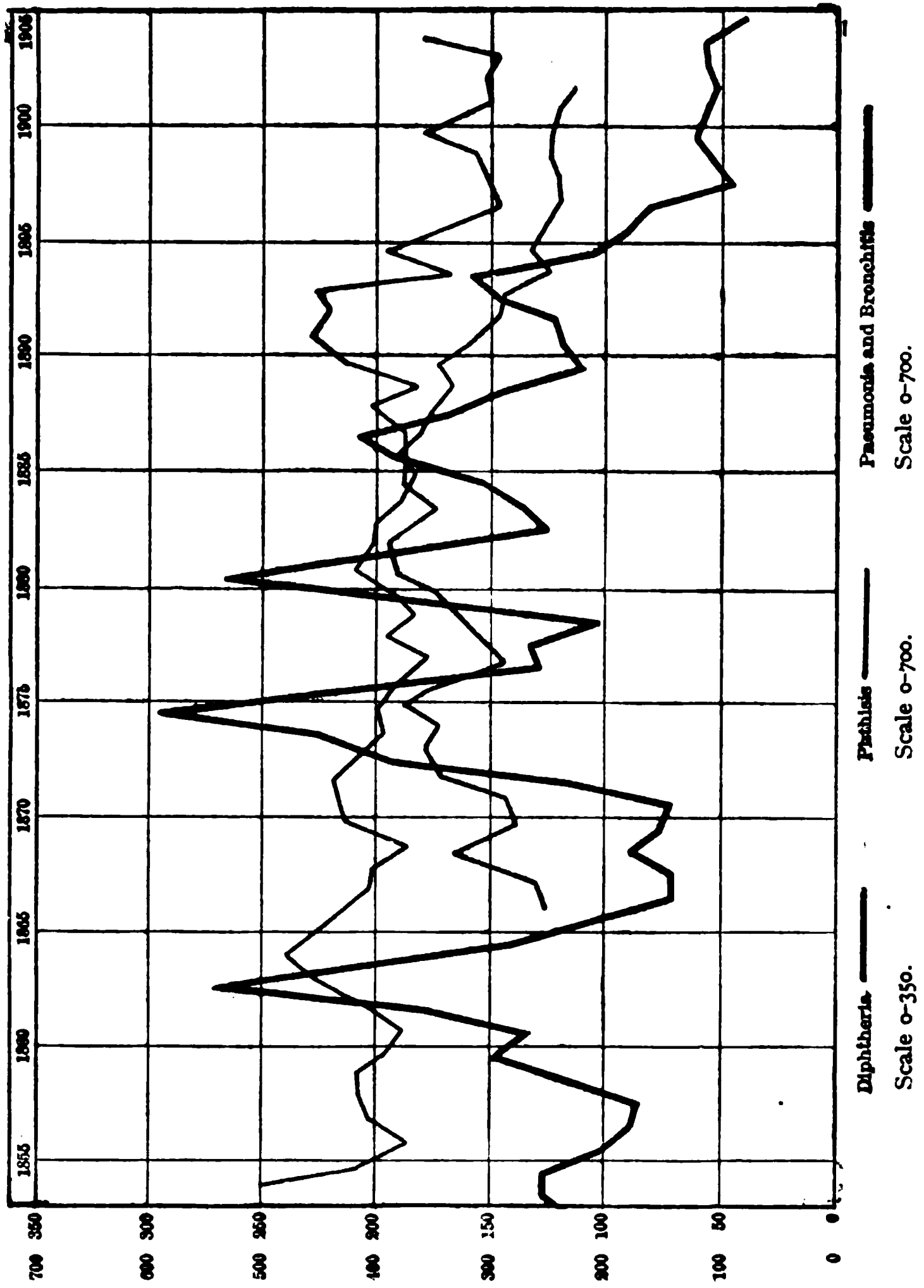


Fig. 3.

A word as to the latter chart:

For the period from 1853-1865 the deaths are all those given in the table on page 40, while from 1866 on, the figures include all the deaths reported as due to diphtheria and croup. During the early part of these years the deaths are undoubtedly understated, for we know that only about two-thirds to three-quarters of all deaths were really reported. For the past thirty-five years, however, the figures are probably very near the actual conditions.

In the case of these two cities, then, we see that there is an irregular epidemic recurrence at intervals varying from about five to ten years. The importance of knowing this cyclical recurrence will be discussed in connection with antitoxin treatment.

MORTALITY AND PREVALENCE OF DIPHTHERIA AT DIFFERENT AGES.

Diphtheria is essentially a children's disease. This fact is emphasized by all writers. To be sure this does not mean that only children are attacked, for the disease is certainly not rare among adults. In fact, some of the earliest modern reports of diphtheria were from cases among the French troops in barracks.

On the whole, it may be safely said that during the first few months there is only a slight disposition for the disease. Toward the end of the first year, however, this disposition grows rapidly, increasing still more rapidly during the second year, so that from then until about the close of the fifth year the disease is at a high level. From then on the disposition drops, at first slowly, then more rapidly, so that from about the 14th or 15th year the disposition is again very slight.

The following figures show the age incidence of 2,711 cases admitted to Baginsky's children's hospital in Berlin from 1890 to 1897 inclusive:

	Cases.	Per cent.	Number who died.	Death rate per 100 admitted.	Death rate in the age class.
0 to 6 months.....	15	.55	4	0.15	26.6
6 months to 1 year.....	69	2.50	36	1.32	52.2
1 to 2 years.....	227	8.30	110	4.05	48.4
2 to 3 years.....	317	11.60	119	4.30	37.5
3 to 4 years.....	354	13.05	121	4.40	34.2
4 to 5 years.....	337	12.40	84	3.09	24.9
5 to 6 years.....	264	9.70	61	2.20	23.1
6 to 7 years.....	280	10.30	61	2.20	21.8
7 to 8 years.....	209	7.70	26	0.95	12.4
8 to 9 years.....	175	6.40	23	0.84	13.1
9 to 10 years.....	146	5.30	19	0.70	13.0
10 to 11 years.....	101	3.70	9	0.33	8.9
11 to 12 years.....	80	2.90	8	0.29	10.0
12 to 13 years.....	65	2.02	7	0.25	10.8
13 to 14 years.....	72	2.60	6	0.22	8.3
	2,711	694	*25.6

*Average.

The following table shows the age distribution of the *fatal cases* in New York City for ten years:

New York City—Deaths from Diphtheria, 1891-1900 Inclusive—Age Distribution.

	Female.	Male.	Total.	Per Cent. of Total.
Under 1 year.	745	920	1,665	9.2
Between 1 and 2.....	1,998	2,265	4,263	23.6
“ 2 and 3.....	1,872	1,945	3,817	21.2
“ 3 and 4.....	1,412	1,488	2,900	16.1
“ 4 and 5.....	960	948	1,908	10.6
Total under 5.	6,987	7,566	14,553	80.6

	Female.	Male.	Total.	Per Cent. of Total.
Between 5 and 10.....	1,681	1,371	3,052	17.0
“ 10 and 15.....	131	110	241	1.3
“ 15 and 20.....	31	29	60	1.7
“ 20 and 25.....	36	22	58	
“ 25 and 35.....	49	46	95	
“ 35 and 45.....	19	22	41	
“ 45 and 55.....	13	16	29	
“ 55 and 65.....	9	7	16	
“ 65 and 75.....	4	4	8	
“ 75 and 85.....	1.7
“ 85 or over.....	1	1	
Total	18,003

In England the age distribution of nearly 70,000 fatal cases compiled by the Registrar General (Lancet, 1878) was as follows:

	Per Cent.
Under 1 year.....	9.0
From 1- 5 years.....	45.0
From 5-10 years.....	26.0
From 10-15 years.....	9.0
From 15-25 years.....	5.0
From 25-45 years.....	3.5
45 years and over.....	2.5

The youngest case of which we can find any record is one reported by Jacobi in a child 9 days old. Forest has recently reported three cases in young infants the ages of which were 19 days, 3 weeks, 11 weeks respectively (Archiv. f. Kinderheilkunde, Vol. XLII., No. 1 and 2.) We have met with two severe pharyngeal cases at an early age, 17 and 21 days respectively.

Sex seems to play no part in this disease, boys and girls are about equally attacked. Of the 2,711 cases above mentioned, 1,311 were boys and 1,400 girls. The mortality among the former was 27.9 per cent.; among the latter 23.2 per cent.

INFECTIVITY AND VIRULENCE.

There is a considerable variation in the case of mortality at different times and in different places. This fact is particularly important in studying the influence of antitoxin on this disease, as will be discussed later.

First of all it is necessary to appreciate clearly that poison production and infectivity are two entirely independent properties. Thus a marked capacity for producing a severe attack of a disease may be associated with a very low degree of infectivity, while the presence of great power of infectivity in an organism or mixture of associated organisms does not imply that the average type of disease produced will be necessarily severe.

Eröss, in studying the statistics from a large number of Hungarian cities, finds that the case mortality in diphtheria is highest at the height of the epidemic, and that as soon as the disease loses its epidemic character, the case mortality drops to a low level. Newsholme, however, shows that this is not always the case, and presents a number of interesting tables which show the reverse behavior. Brownlee in an excellent paper on this subject states that the disease is as a rule most fatal in England in towns where it is least prevalent.

RESULTS OF ANTITOXIN TREATMENT.

A period of more than ten years having elapsed since the introduction of diphtheria antitoxin, it seems advisable to study the effect this treatment has had on the mortality from diphtheria.

On looking over the literature one finds that such studies have been made a number of times, and that many of them are exceedingly valuable. Nevertheless objections have been made, particularly by opponents of serum treatment, that such statistics are open to grave sources of error, and that the apparent improvement is only temporary. Instead of this contention receiving support as the antitoxin treatment extends over a longer period and becomes more general we find that in spite of temporary fluctuations the trend is always toward a lower mortality.

In the early period of antitoxin administration (in 1896) the American Pediatric Society collected reports on 5,794 cases of diphtheria treated with antitoxin. These showed an average mortality of 12.3 per cent.

Guerard, in Bulletin No. 3 of New York Health Department, collected reports of 9,893 cases treated with the serum, with an average mortality of 18.3 per cent. Of these cases 7,277 in which the mortality was 20 per cent. were returned by 53 hospitals; the reports from the same hospitals gave as their previous mortality an average of 44.3 per cent.

In the City of Boston, Ernst reports 1,156 cases treated by the serum, with 165 deaths, a mortality of 14.2 per cent. The report by McCollom from the diphtheria wards of the Boston City Hospital shows even better results. Of 844 cases treated by the serum, there were 96 deaths, a mortality of 11 per cent.; the previous mortality in the same institution without serum was 40 per cent.

Siegert tabulated the cases from 23 hospitals for children in Europe from 1890 to 1898 inclusive. Omitting 1894, the transition year, his figures are as follows:

Period.	Cases Treated.	Deaths.	Mortality.
Before serum, 1890-1893.....	16,585	6,889	41.3
After serum, 1895-1898.....	20,181	3,309	16.4

In the "Statistische Monatsschrift," 1902, quoted by Eröss (Jahrb. f. Kinderheilk., 1904, No. 60, p. 593), we find that in Austria in 1896-1899 of the patients treated with serum 14.5 to 15.1 per cent. died, while of those not treated 36.4 to 39.8 per cent. died, viz., 2½ times as many.

Bokay (Jahrb. f. Kinderheilk., 1904), in referring to diphtheria in Buda Pest, says that in 1891 out of 2,952 cases of diphtheria there were 914 deaths, 32.5 per cent. In the year 1903 out of 2,293 cases only 309 died, 13.5 per cent. By merely placing these two years side by side, he says there is enough statistical material to show the great value of serum treatment.

The above figures may be taken as an example of the "case-mortality method." It is open to several objections, chief among which is the

fact that what is now called diphtheria and membranous croup may or may not have been so designated formerly.

Operative Cases.

A comparison of the mortality in the operative cases is of particular interest, since these are always diphtherias of a severe type.

The following figures are compiled from Lovett and Munro,¹ Holt,² McCollom³ and from our own official records:

Without Serum.

Intubations.	Without Serum.		
	Total Cases.	Died.	Mortality.
German authors.....	5,795	3,944	69 per cent.
German hospitals.....	3,063	2,124	70 “
British authors.....	433	295	69 “
French authors.....	9,242	6,834	76 “
American authors—private practice.....	5,625	3,848	68 “
Various countries.....	1,993	1,336	68 “
Boston City Hospital (23 years).....	71 “

With Serum.

Intubations.	With Serum.		
	Total Cases.	Died.	Mortality.
Welch—European hospitals.....	342	112	29.8 per cent.
American Pediatric Society Report, private practice.....	533	138	25.9 “
McCollom—Hospital cases—Boston.....	1,553	683	44.0 “
New York Health Department, as below.....	1,660	723	43.5 “

New York Health Department—	Cases.	Deaths.
Tenement service, 1895-7.....	144	56
Tenement service, 1902-4.....	133	39
Diphtheria Hospital, 1901-5.....	1,341	614
Outside physicians, 1895-6.....	42	14
Total.....	1,660	723

¹ Lovett & Munroe. American Journ. of Med. Sciences, 1887. Vol. XCIV., p. 160.
² Infancy and Childhood. First edition, page 189.
³ John McCollom. Boston Med. & Surg. Journal, Vol. CLII., No. 22, 1905.

The value of intubation in this disease is well shown by the following table of treatment with tracheotomy, but without serum.

	Total Cases.	Died.	Mortality.
German authors.....	5,795	3,944	69 per cent.
German hospitals.....	3,063	2,124	70 "
British authors.....	433	295	69 "
French authors.....	9,242	6,834	76 "
Various countries.....	1,993	1,336	68 "
American authors.....	1,327	1,015	77 "
	21,853	15,552	72 per cent.

The Simultaneous Observation of Cases Treated Without and With Antitoxin.

An absolutely ideal method is one made use of by Fibinger (cited by Faber, Jahrb. f. Kinderheilk 1904, No. 59). In this at the same time every other case was treated with antitoxin. He had 239 cases with antitoxin with mortality of 8—3 per cent.; 245 cases without antitoxin with mortality of 30—12 per cent.

This method however for obvious reasons is not available at this day. We once observed a similar test at the Willard Parker Hospital. The difference in the behavior of the cases was so greatly in favor of the antitoxin that the test was stopped and all cases put on antitoxin.

The Absolute Number of Deaths from Diphtheria Before and After the Introduction of Antitoxin.

Perhaps the least objectionable of all methods at present available is a comparison over a series of years of the absolute number of deaths per 100,000 for a long period of years before and after the introduction of antitoxin. Such figures moreover must be collected only from such cities where reliable statistics have been kept during the entire period and any extraneous factors, such as changes in nomenclature, the presence of epidemics must be known.

It was stated above that the statistics must be from a long period of years. While this of course is true for all kinds of statistics it is par-

ticularly important in diphtheria in which mortality figures move up and down irregularly in large waves. These irregularities, however, only become apparent when a considerable number of years is gone over. To give an example, in Baltimore in the six years ending 1882, the average of deaths per 100,000 from diphtheria and croup was always above 14 and reached 200 or over in three of those years. In the seven years following, the mortality fell sharply and continuously until it reached its ebb point in 1889, when it was 52 per 100,000, and yet no difference in treatment was introduced in 1883.

One must therefore be careful not merely to take readings which constitute part of an epidemic, unless due allowance be made for this fact. And in order to appreciate what an epidemic is, one must know the average number of deaths for many years back.

Statistics of this kind ought furthermore to be taken mainly from the large cities, for reports of deaths are usually but indifferently kept in the rural districts. Thus in some states the cause of death was often certified to the health authorities by the town supervisor, so that it happened that "sore inside," "chronic running sores" were occasionally given as causes of death. In some of these States only about two-thirds of the deaths have been regularly reported.

In the following pages statistics have been collected from a number of large cities on the continent of Europe, in Great Britain, and in the United States.

For purposes of comparison it will be perfectly fair to take the combined mortality from diphtheria and croup for the years of the preantitoxin period and compare this with that of the years since the introduction of antitoxin.

New York City (Present Boroughs Manhattan and Bronx).

Year.	Population.	Deaths, Diphtheria and Croup.	Per 100,000
1851.....	538,490	636	118
1852.....	560,607	641	114
1853.....	583,632	673	115
1854.....	607,603	772	127
1855.....	632,559	804	127
1856.....	664,980	682	102
1857.....	699,062	622	90
1858.....	734,892	644	87
1859.....	772,558	894	116
1860.....	812,154	1,211	149
1861.....	794,905	1,072	135
1862.....	778,023	1,383	179
1863.....	761,500	2,041	268
1864.....	745,327	1,603	215
1865.....	729,498	1,016	140
1866.....	767,979	803	104
1867.....	808,489	585	72
1868.....	851,137	616	72
1869.....	896,034	813	90
1870.....	943,300	729	77
1871.....	955,921	704	73
1872.....	968,710	1,121	116
1873.....	981,676	1,883	192
1874.....	1,030,607	2,259	219
1875.....	1,044,396	3,087	295
1876.....	1,075,532	2,277	211
1877.....	1,107,597	1,423	128
1878.....	1,140,617	1,506	132
1879.....	1,171,621	2,300	190
1880.....	1,209,268	1,193	101
1881.....	1,244,511	3,287	264
1882.....	1,280,857	2,254	184
1883.....	1,318,264	1,653	125
1884.....	1,356,764	1,838	136
1885.....	1,396,388	2,180	158
1886.....	1,437,170	2,695	188

Year.	Population.	Deaths, Diphtheria and Croup.	Per 100,000
1887.....	1,479,143	3,056	206
1888.....	1,522,341	2,553	167
1889.....	1,566,801	2,291	146
1890.....	1,612,559	1,783	110
1891.....	1,659,654	1,970	118
1892.....	1,708,124	2,105	123
1893.....	1,758,010	2,558	145
1894.....	1,895,353	2,870	158
1895.....	1,873,201	1,976	105
1896.....	1,906,139	1,763	91
1897.....	1,940,553	1,590	81
1898.....	1,976,572	923	46
1899.....	2,014,330	1,085	53
1900.....	2,055,714	1,276	62
1901.....	2,118,209	1,227	58
1902.....	2,182,836	1,142	53
1903.....	2,249,680	1,232	56
1904.....	2,318,831	1,272	57
1905.....	2,390,382	860	38

Antitoxin laboratory established in fall of 1894. Free distribution to the poor begun early in 1895.

The deaths classed in this table as due to diphtheria from 1851-1865 are only approximately correct. They include all the deaths during the period mentioned (1851-1865 inclusive), which were returned under any of the following heads: Angina, Croup, Diphtheria, Inflammation of Throat, Inflammation of Tonsil, Quinsey, Sprue, Ulceration of Throat. It is estimated that prior to 1851 not more than two-thirds of the deaths were registered. Since then conditions have gradually improved. It must also be remembered that scarlet fever and diphtheria were often confounded in these earlier days.

Brooklyn.

Year.	Population.	Deaths, Diphtheria and Croup.	Per 100,000
1878.....	536,561	861	162
1879.....	551,397	939	170
1880.....	566,663	1,538	270
1881.....	585,017	1,607	275
1882.....	603,926	965	160
1883.....	623,422	727	116
1884.....	643,487	665	103
1885.....	664,126	832	125
1886.....	707,092	1,185	167
1887.....	730,712	1,453	200
1888.....	755,145	1,265	164
1889.....	856,321	1,467	180
1890.....	894,024	1,283	152
1891.....	933,398	1,180	135
1892.....	970,046	1,137	126
1893.....	996,715	978	105
1894.....	1,060,000	1,660	173
1895.....	1,100,000	1,454	146
1896.....	1,125,000	1,310	127
1897.....	1,162,749	998	94
1898.....	1,197,100	745	68
1899.....	1,231,548	744	65
1900.....	1,169,000	863	73
1901.....	1,205,000	732	60
1902.....	1,243,000	762	61
1903.....	1,281,000	803	62
1904.....	1,321,000	706	53
1905.....	1,362,000	594	43

Antitoxin distributed gratis from the time of Brooklyn's consolidation with New York, in 1896.

Boston.

Year.	Population.	Deaths, Diphtheria and Croup.	Deaths Per 100,000.
1876.....	346,004	720	208
1877.....	350,138	471	134
1878.....	354,322	569	160
1879.....	358,554	545	152
1880.....	362,839	774	213
1881.....	368,190	802	217
1882.....	373,620	575	153
1883.....	379,129	608	160
1884.....	384,720	487	126
1885.....	390,393	459	117
1886.....	401,374	423	105
1887.....	412,663	410	99
1888.....	424,274	589	138
1889.....	436,208	683	156
1890.....	448,477	462	103
1891.....	457,772	285	62
1892.....	467,270	481	102
1893.....	476,270	546	114
1894.....	486,830	878	180
1895.....	501,083	588	117
1896.....	516,305	516	98
1897.....	528,912	411	77
1898.....	541,827	170	31
1899.....	555,057	277	49
1900.....	560,892	537	95
1901.....	573,579	353	61
1902.....	586,533	225	38
1903.....	600,929	214	35
1904.....	614,522	206	33
1905.....	595,000	132	22

REMARKS—Antitoxin laboratory established 1895. First serum issued September 1, 1905. Free distribution of antitoxin to poor since 1896. Its use was quite general even from the first. At present about 60 per cent. of all cases of diphtheria are treated in the hospital and receive antitoxin.

Philadelphia.

Year.	Population.	Deaths from Diphtheria.	Croup.	Total.	Deaths Per 100,000	
1878.....	825,000	464	388	854	103	
1879.....	835,000	321	291	612	72	
1880.....	846,980	323	303	626	74	
1881.....	868,000	457	317	774	90	
1882.....	886,509	933	466	1,399	158	
1883.....	907,041	1,006	500	1,506	166	
1884.....	927,995	680	589	1,269	135	
1885.....	949,432	600	753	1,353	143	
1886.....	971,363	411	650	1,061	109	
1887.....	993,801	416	442	858	86	
1888.....	1,016,758	350	273	623	61	
1889.....	1,046,964	375	352	727	70	
1890.....	1,040,964	528	415	943	90	
1891.....	1,069,264	918	444	1,362	129	
1892.....	1,092,168	1,435	272	1,707	156	
			Mem- branous.	Plain.		
1893.....	1,115,562	916	273	79	1,238	110
1894.....	1,139,457	1,407	348	56	1,451	128
1895.....	1,163,864	1,020	329	49	1,398	120
1896.....	1,168,864	862	293	46	1,201	103
1897.....	1,214,256	1,231	243	40	1,514	125
1898.....	1,240,266	998	156	21	1,175	94
1899.....	1,266,832	849	144	21	1,014	80
1900.....	1,293,697	898	144	25	1,163	82
1901.....	1,321,408	525	118	18	661	50
1902.....	1,349,712	435	80	16	531	40
1903.....	1,378,624	521	87	22	631	46
1904.....	1,408,154	458	83	7	548	38
1905.....	1,438,000	462	32

Philadelphia has distributed antitoxin free of charge for use among the poor since 1896. For several years after this, however, its use was not general. Since about 1900, however, its use has extended, as is shown by the following figures there were supplied: 1896, 900 doses; 1897, 2,334 doses; 1898, 3,367 doses; 1899, 2,976 doses; 1900, 5,233 doses; 1901, 5,374 doses; 1902, 5,771 doses; 1903, 7,342 doses; 1904, 10,298 doses.

Baltimore.

Year.	Population.	Deaths from Diph- theria and Membrane Croup.	Croup.	Total.	Per 100,000.
1877.....	311,275	455	157	612	197
1878.....	318,182	303	149	452	142
1879.....	325,139	298	186	484	150
1880.....	332,313	293	173	466	140
1881.....	339,649	639	242	881	260
1882.....	347,142	707	222	929	265
1883.....	354,832	591	201	792	143
1884.....	362,668	343	127	370	102
1885.....	370,696	252	148	300	81
1886.....	378,903	190	128	318	84
1887.....	387,300	149	153	302	78
1888.....	395,899	118	98	216	54
1889.....	404,498	155	53	208	51
1890.....	413,671	274	45	319	77
1891.....	426,917	350	44	394	92
1892.....	440,163	381	47	428	98
1893.....	453,409	185	25	310	69
1894.....	466,655	198	33	231	50
1895.....	479,907	265	45	310	65
1896.....	493,147	249	32	281	57
1897.....	500,000 estimated	347	13	360	72
1898.....	505,000	" 362	50	412	81
1899.....	509,000	" 312	...	312	61
1900.....	513,000	" 267	12	279	54
1901.....	518,000	"	171	33
1902.....	525,000	"	130	25
1903.....	533,000	"	160	30
1904.....	541,000	"	115	21
1905.....	550,000	"	112	20

Pittsburg.

Year.	Population.	Deaths, Diphtheria.	Croup.	Total.	Per 100,000.
1878.....	145,000	483	12	495	340
1879.....	150,000	354	5	359	240
1880.....	156,381	311	missing	311+	200
1881.....	165,000	210	"	210+	130
1882.....	170,000	185	"	185+	109
1883.....	175,000	170	"	170+	98
1884.....	185,000	321	"	321+	180
1885.....	202,559	243	"	243+	120
1886.....	205,000	249	11	260	127
1887.....	210,000	281	103	384	183
1888.....	220,000	126	73	199	90
1889.....	230,000	333		333	138
1890.....	238,617	394		394	164
1891.....	247,000	388		388	157
1892.....	255,000	372		372	145
1893.....	264,000	233		233	88
1894.....	272,000	175		175	64
1895.....	275,000	187		187	68
1896.....	282,500	183		183	64
1897.....	287,500	135		135	46
1898.....	298,772	89		89	30
1899.....	306,115	89		89	29
1900.....	321,616 census	147		147	46
1901.....	330,000 approximate	165		165	50
1902.....	340,000	"	170	170	50
1903.....	350,000	"	215	215	60
1904.....	360,000	"	166	166	46
1905.....	370,000	"	...	98	27

Serum laboratory opened in the fall of 1895.

Antitoxin distribution, free and unrestricted, began about September, 1895. Its use became general only during October and later, 1896.

London.

Year.	Population.	Deaths, Diphtheria and Croup.	Death Rate per 100,000.
1875.....	3,482,306	1,312	37.7
1876.....	3,538,246	987	27.9
1877.....	3,595,085	855	23.8
1878.....	3,652,837	1,150	31.5
1879.....	3,711,517	1,169	31.5
1880.....	3,771,139	1,108	29.4
1881.....	3,824,980	1,346	35.2
1882.....	3,862,956	1,707	44.2
1883.....	3,901,309	1,771	45.4
1884.....	3,940,042	1,698	43.1
1885.....	3,979,160	1,580	39.7
1886.....	4,018,666	1,374	34.2
1887.....	4,058,565	1,481	36.5
1888.....	4,098,860	1,800	43.9
1889.....	4,139,555	1,784	43.1
1890.....	4,180,654	1,885	45.1
1891.....	4,223,720	1,858	44.0
1892.....	4,269,634	2,241	52.5
1893.....	4,312,623	3,454	80.1
1894.....	4,351,501	2,873	66.0
1895.....	4,387,248	2,488	56.7
1896.....	4,419,411	2,804	63.0
1897.....	4,447,907	2,345	52.0
1898.....	4,472,664	1,823	40.8
1899.....	4,493,617	2,028	45.0
1900.....	4,510,711	1,620	36.0
1901.....	4,544,983	1,385	35.0
1902.....	4,579,110	1,199	26.0
1903.....	4,613,812	778	17.0
1904.....	4,648,950	754	16.0
1905.....	4,700,000	573	12.2

Liverpool.

Year.	Population.	Deaths, Diphtheria and Croup.	Per 100,000.
1878.....	534,000	299	56
1879.....	541,000	201	37
1880.....	548,000	238	43
1881.....	552,508	193	35
1882.....	549,000	206	38
1883.....	546,000	223	41
1884.....	543,000	252	46
1885.....	540,000	346	64
1886.....	537,000	267	50
1887.....	534,000	209	39
1888.....	531,000	174	33
1889.....	527,000	186	35
1890.....	523,000	211	41
1891.....	517,980	119	23
1892.....	518,000	109	21
1893.....	518,000	85	17
1894.....	518,000	127	24
1895.....	638,291 *	170	27
1896.....	641,000	202	32
1897.....	663,633	149	22
1898.....	669,243	164	23
1899.....	674,912	242	36
1900.....	680,628	183	30
1901.....	686,332	209	30
1902.....	710,337 *	241	34
1903.....	716,810	177	24
1904.....	723,430	214	28
1905.....	723,000	214	29

* City boundaries extended.

Glasgow.

Year.	Population.	Deaths, Diphtheria and Croup.	Per 100,000.
1881.....	511,415 census
1882.....	517,000 approximate
1883.....	522,000	"	...
1884.....	528,000	"	...
1885.....	533,000	"	...
1886.....	538,000	"	211 39.1
1887.....	543,000	"	291 53.6
1888.....	548,000	"	274 50.0
1889.....	553,000	"	298 54.0
1890.....	558,000	"	203 36.3
1891.....	565,710 census	194	34.4
1892.....	669,059	249	37.2
1893.....	677,883	310	45.8
1894.....	686,820	306	45.1
1895.....	695,876	185	26.6
1896.....	705,052	137	19.4
1897.....	714,919	145	20.3
1898.....	724,349	132	18.2
1899.....	733,903	123	16.8
1900.....	743,963	149	20.0
1901.....	764,467	123	16.1
1902.....	775,601	127	16.4
1903.....	787,897	118	15.0
1904.....	798,357	104	13.0
1905.....	810,000	48	6.0

Berlin.

Year.	Population.	Deaths, Diphtheria and Croup.	Per 100,000.
1878.....	1,033,632	1,446	140
1879.....	1,065,440	1,355	127
1880.....	1,107,100	1,422	129
1881.....	1,138,700	1,778	156
1882.....	1,174,293	2,134	181
1883.....	1,207,114	2,932	243
1884.....	1,225,065	2,640	215
1885.....	1,299,207	2,007	154
1886.....	1,337,798	1,688	126
1887.....	1,376,389	1,404	112
1888.....	1,414,980	1,100	79
1889.....	1,453,571	1,284	88
1890.....	1,492,162	1,591	107
1891.....	1,609,415	1,075	66
1892.....	1,662,237	1,414	85
1893.....	1,714,938	1,643	95
1894.....	1,767,639	1,430	81
1895.....	1,669,138	1,000	59
1896.....	1,695,726	569	33
1897.....	1,708,499	547	32
1898.....	1,728,201	664	38
1899.....	1,747,903	655	37
1900.....	1,864,203	563	30
1901.....	1,913,528	513	26
1902.....	1,955,837	231	11
1903.....	1,998,146	245	12
1904.....	2,040,455	354	17
1905.....	2,026,000	310	15

Muenchen.

Year.	Population.	Deaths, Diphtheria and Croup.	Per 100,000.
1878.....	222,000	298	130
1879.....	225,000	293	130
1880.....	229,854	367	160
1881.....	233,000	394	169
1882.....	238,000	263	110
1883.....	240,000	272	113
1884.....	242,000	182	75
1885.....	259,318	176	68
1886.....	265,710	223	86
1887.....	272,102	204	75
1888.....	728,494	264	36
1889.....	284,886	401	141
1890.....	291,278	303	104
1891.....	352,718	340	96
1892.....	372,418	308	83
1893.....	386,202	258	66
1894.....	399,986	284	73
1895.....	402,459	208	51
1896.....	412,000	185	44
1897.....	425,087	182	42
1898.....	436,430	181	41
1899.....	452,248	104	23
1900.....	499,932	110	22
1901.....	510,044	80	20
1902.....	527,379	68	12
1903.....	544,714	74	13
1904.....	562,049	89	15
1905.....	579,000	84	15

Königsberg.

Year.	Population.	Deaths, Diphtheria and Croup.	Per 100,000.
1878.....	128,096	263	205
1879.....	132,028	197	150
1880.....	140,932	179	128
1881.....	141,560	262	178
1882.....	146,678	352	241
1883.....	150,350	287	191
1884.....	154,000	249	161
1885.....	150,297	286	190
1886.....	152,345	154	101
1887.....	154,393	179	120
1888.....	156,441	122	79
1889.....	158,489	211	133
1890.....	159,537	194	122
1891.....	162,739	92	50
1892.....	164,996	173	105
1893.....	167,099	179	107
1894.....	169,202	162	95
1895.....	171,497	86	50
1896.....	174,094	91	52
1897.....	176,595	74	42
1898.....	178,821	17	9
1899.....	181,047	16	9
1900.....	185,987	37	20
1901.....	191,429	32	16
1902.....	194,766	224	*115
1903.....	198,103	121	61
1904.....	201,440	86	42
1905.....	197,000	44	22

* Serious epidemic ; antitoxin not extensively used owing to expense ; no gratuitous distribution by city.

Hamburg.

Year.	Population.	Deaths, Diphtheria and Croup.	Per 100,000.
1878.....	371,843	244	65
1879.....	277,068	256	68
1880.....	410,127	286	70
1881.....	416,295	282	67
1882.....	416,819	329	79
1883.....	435,964	336	77
1884.....	449,414	443	98
1885.....	462,278	509	110
1886.....	474,370	580	122
1887.....	486,462	567	117
1888.....	498,554	466	94
1889.....	510,646	475	93
1890.....	522,738	352	67
1891.....	571,689	216	38
1892.....	594,273	226	38
1893.....	612,933	362	59
1894.....	636,194	385	60
1895.....	618,945	132	21
1896.....	631,660	95	15
1897.....	642,131	113	17
1898.....	652,606	100	15
1899.....	663,073	109	16
1900.....	673,794	115	17
1901.....	715,093	116	16
1902.....	731,130	183	25
1903.....	747,167	160	21
1904.....	763,204	130	17
1905.....	790,000	84	10

Dresden.

Year.	Population.	Deaths, Diphtheria and Croup.	Per 100,000.
1878.....	210,377	217	103
1879.....	215,400	159	74
1880.....	220,216	284	130
1881.....	223,100	381	170
1882.....	227,250	534	235
1883.....	233,600	495	212
1884.....	236,000	467	200
1885.....	243,980	342	140
1886.....	249,034	416	167
1887.....	254,088	330	130
1888.....	259,142	268	104
1889.....	264,198	268	101
1890.....	269,250	253	94
1891.....	297,585	256	91
1892.....	286,160	384	134
1893.....	304,519	370	121
1894.....	311,168	349	112
1895.....	330,172	170	51
1896.....	342,300	107	31
1897.....	368,485	115	31
1898.....	379,268	85	22
1899.....	388,587	88	23
1900.....	391,927	58	15
1901.....	404,773	57	14
1902.....	416,919	63	15
1903.....	518,405	76	16
1904.....	533,017	108	20
1905.....	530,000	74	14

Breslau.

Year.	Population.	Deaths, Diphtheria and Croup.	Per 100,000.
1878.....	267,000	106	40
1879.....	270,000	105	39
1880.....	272,000	135	50
1881.....	275,000	158	57
1882.....	280,000	163	60
1883.....	287,000	323	113
1884.....	295,000	239	81
1885.....	297,413	218	73
1886.....	302,759	280	92
1887.....	308,105	497	125
1888.....	313,451	495	160
1889.....	318,797	382	120
1890.....	324,143	362	111
1891.....	339,318	323	95
1892.....	346,442	257	74
1893.....	353,551	409	116
1894.....	360,660	314	87
1895.....	370,038	242	65
1896.....	378,089	125	33
1897.....	385,198	98	25
1898.....	398,415	93	24
1899.....	405,362	94	23
1900.....	412,959	49	12
1901.....	427,833	71	17
1902.....	436,618	87	20
1903.....	445,403	96	21
1904.....	454,188	85	18
1905.....	465,000	92	20

Cologne.

Year.	Population.	Deaths, Diphtheria and Croup.	Per 100,000.
1878.....	138,836	45	40
1879.....	140,104	55	39
1880.....	144,735	82	57
1881.....	146,000	78	53
1882.....	146,767	66	45
1883.....	148,640	55	37
1884.....	151,500	29	19
1885.....	160,015	70	44
1886.....	163,341	87	53
1887.....	166,667	79	48
1888.....	169,993	99	57
1889.....	261,105	89	38
1891.....	267,152	173	90
1892.....	286,230	255	120
1893.....	295,059	359	170
1894.....	303,508	517	135
1895.....	311,597	421	54
1896.....	319,000	173	44
1897.....	326,800	154	45
1898.....	332,773	150	50
1899.....	341,651	172	31
1900.....	349,628	110	12
1901.....	368,006	46	27
1902.....	378,541	101	30
1903.....	399,126	117	28
1904.....	409,322	113	28
1905.....	425,000	95	22

Frankfurt.

Year.	Population.	Deaths, Diphtheria and Croup.	Per 100,000.
1878.....	122,292	79	65
1879.....	126,000	52	41
1880.....	136,831	33	24
1881.....	139,710	46	33
1882.....	140,000	53	38
1883.....	143,300	41	29
1884.....	144,600	73	50
1885.....	153,038	86	56
1886.....	156,577	123	80
1887.....	160,116	230	143
1888.....	163,655	162	99
1889.....	167,194	228	130
1890.....	170,733	285	168
1891.....	182,804	285	156
1892.....	188,050	278	149
1893.....	193,440	268	134
1894.....	198,238	208	105
1895.....	226,000	67	30
1896.....	233,000	46	20
1897.....	238,684	29	12
1898.....	244,808	23	9
1899.....	250,932	46	18
1900.....	264,645	33	12
1901.....	294,052	30	10
1902.....	302,731	44	14
1903.....	311,410	38	12
1904.....	320,089	49	15
1905.....	330,000	30	9

Vienna.

Year.	Population.	Deaths, Diphtheria and Croup.	Per 100,000
1880.....	721,016	597	83
1881.....	741,208	539	73
1882.....	749,919	522	70
1883.....	750,762	360	48
1884.....	759,849	342	45
1885.....	769,889	464	60
1886.....	780,066	546	70
1887.....	790,381	455	58
1888.....	800,836	521	65
1889.....	811,434	513	63
1890.....	822,176	536	65
1891.....	1,378,530	1,311	95
1892.....	1,406,933	1,580	112
1893.....	1,435,931	1,615	112
1894.....	1,465,637	1,679	114
1895.....	1,495,764	710	47
1896.....	1,526,623	621	40
1897.....	1,551,129	575	37
1898.....	1,590,295	520	32
1899.....	1,623,134	463	29
1900.....	1,656,662	306	18
1901.....	1,691,996	387	22
1902.....	1,726,604	438	25
1903.....	1,744,177	426	24
1904.....	1,797,992	386	21
1905.....	1,877,000	254	13

Paris.

Year.	Population.	Deaths, Diphtheria.	Per 100,000.
1880.....	2,225,000	2,048	92
1881.....	2,239,938 census	2,211	90
1882.....	2,244,000	2,244	100
1883.....	2,248,000	1,781	79
1884.....	2,251,000	1,928	85
1885.....	2,256,000	1,655	73
1886.....	2,260,945 census	1,512	67
1887.....	2,280,000	1,585	70
1888.....	2,300,000	1,729	75
1889.....	2,340,000	1,706	73
1890.....	2,380,000	1,668	70
1891.....	2,424,705	1,531	63
1892.....	2,444,000	1,403	59
1893.....	2,460,000	1,266	51
1894.....	2,480,000	1,009	40
1895.....	2,500,000	421	17
1896.....	2,511,629 census	444	18
1897.....	2,540,000	298	12
1898.....	2,570,000	259	10
1899.....	2,600,000	336	13
1900.....	2,630,000	294	11
1901.....	2,660,494 census	628	24
1902.....	2,690,000	529	20
1903.....	2,730,000	396	15
1904.....	2,780,000	250	9
1905.....	2,820,000	163	6

COMBINED STATISTICS.

Deaths and Death Rates from Diphtheria and Croup—New York, Brooklyn, Boston, Pittsburg, Baltimore, Philadelphia, Berlin, Cologne, Breslau, Dresden, Hamburg, Königsberg, Munich, Vienna, London, Glasgow, Liverpool, Paris, Frankfurt.

Year.	Population.	Deaths, Diphtheria and Croup.	Per 100,000.
1878.....	10,000,508	8,185	81.8
1879.....	10,188,268	7,205	70.7
1880.....	13,401,394	11,526	86.0
1881.....	13,642,366	13,897	101.9
1882.....	13,857,726	14,075	101.6
1883.....	14,049,727	13,721	97.6
1884.....	14,353,102	11,930	83.2
1885.....	14,544,489	12,399	85.2
1886.....	15,337,513	12,385	80.8
1887.....	15,617,867	12,721	79.5
1888.....	16,217,823	11,798	72.7
1889.....	16,300,948	12,247	75.1
1890.....	16,526,135	11,059	66.9
1891.....	17,689,146	12,389	70.0
1892.....	18,330,737	14,200	77.5
1893.....	18,467,970	15,726	80.4
1894.....	19,033,902	15,125	79.9
1895.....	19,143,188	10,657	55.6
1896.....	19,489,682	9,651	49.5
1897.....	19,800,629	8,942	45.2
1898.....	20,037,918	7,170	35.7
1899.....	20,358,857	7,256	35.6
1900.....	20,764,614	6,791	32.7
1901.....	20,874,572	6,104	29.2
1902.....	21,552,398	5,630	26.1
1903.....	21,865,299	5,117	23.4
1904.....	22,532,848	4,917	21.8
1905.....	22,790,000	4,323	18.9

The statistics for Vienna do not begin until 1880; those for Glasgow until 1886. The figures for Paris are those of diphtheria only.

We have plotted the above figures in the form of curves which we present herewith :

Fig. 4.

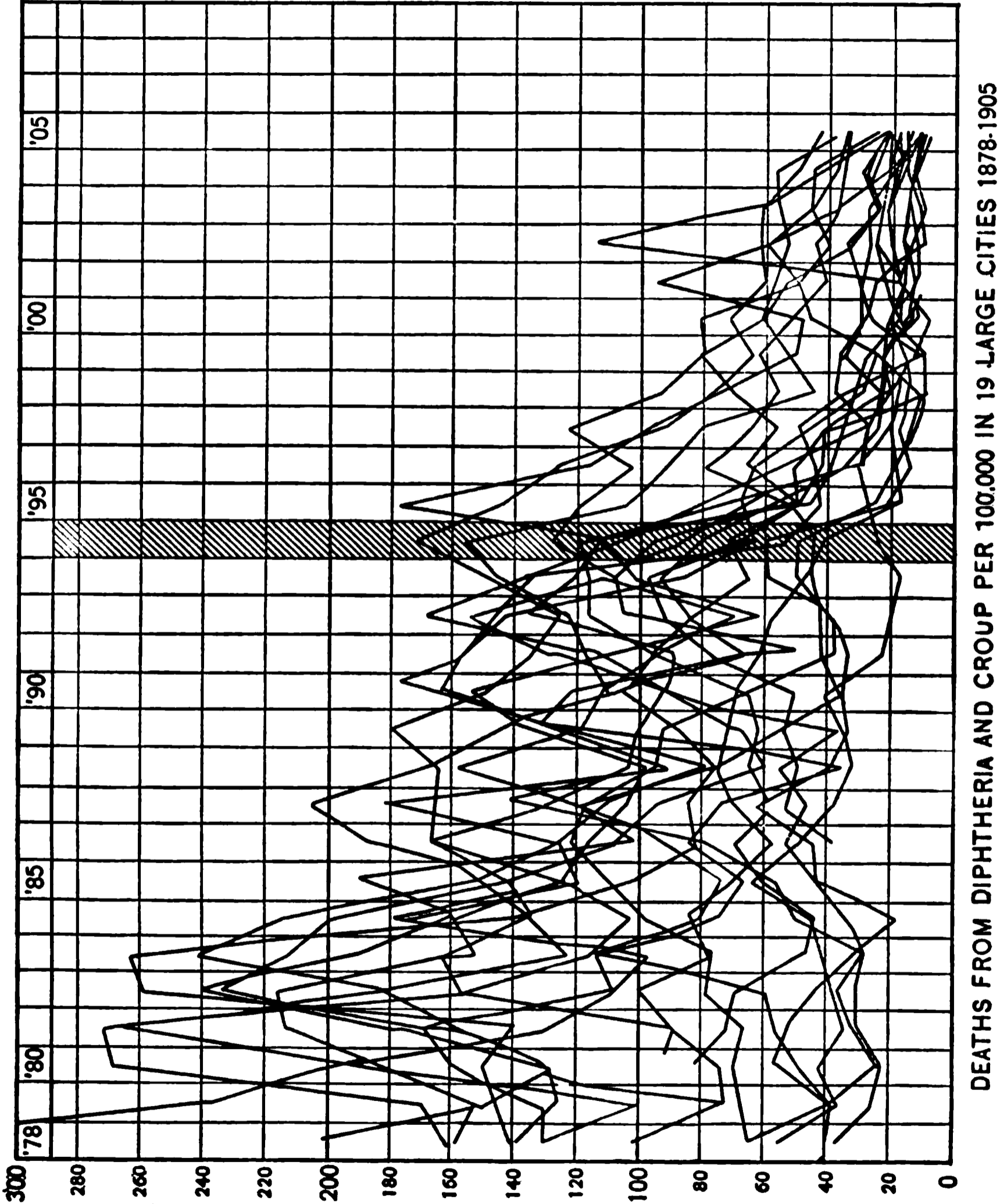
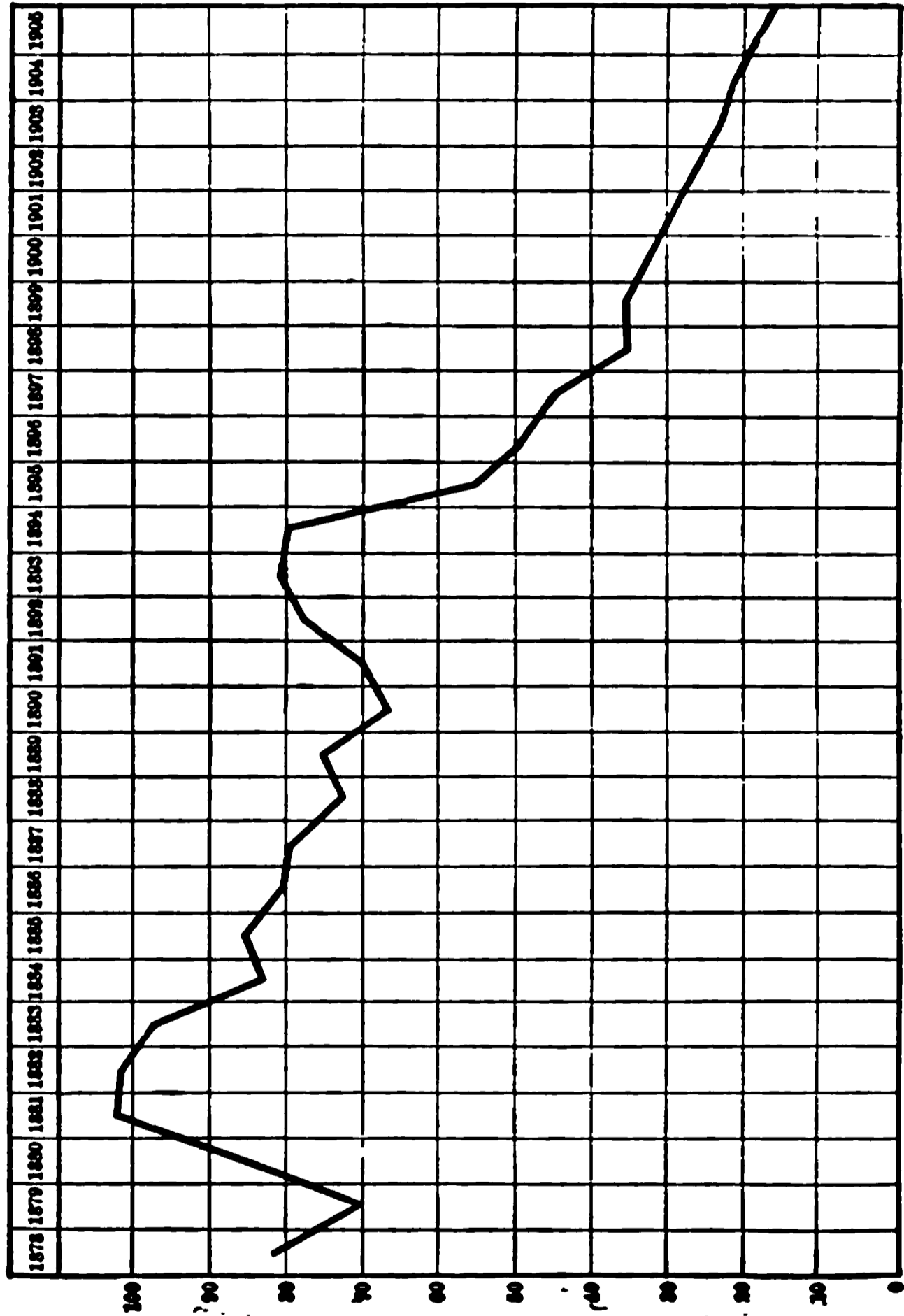


Fig. 5.



COMBINED CURVE FOR THE NINETEEN CITIES.

The two charts, one of nineteen cities combined and the other of the same cities treated singly, present in a very striking manner the great reduction in the number of deaths from diphtheria which occurred in 1895, the year of the beginning of the general use of antitoxin. A study of the prevalence of diphtheria during 1895 shows that there was no appreciable reduction in the cases in these cities merely of the deaths. Of the 19 cities only two show a slight increase while the others show a great reduction. The combined charts show in a most impressive manner how as the antitoxin treatment became more general the deaths became less and less until in 1904 they were but one-quarter of what they were in 1894. With later years, along with the lessened death rate, there have been less cases, as immunization has been more employed and the shortening of the period of disease in treated cases has prevented to some extent liability to infection. It is interesting to contrast diphtheria with phthisis in which along other lines such successful warfare has been waged. The reduction in the mortality in diphtheria has been more than double that in phthisis. (See figure 3.)

While the preceding tables serve as an accurate index of the value of serum treatment, they cannot be used for a comparison of the mortalities in different cities without being subjected to a correction for age distribution. Diphtheria being a disease especially of childhood, it is obvious that the relative proportion of children in a community will greatly affect the death rate from this disease. Since our purpose in presenting the tables has not been to make such comparisons we shall content ourselves merely with calling attention to this factor. The following table kindly compiled for us by Dr. Roger S. Tracy, formerly Registrar of the Department, will give some idea of the influence exerted by age distribution. In compiling this he has taken the total number of deaths from diphtheria and croup in New York in 1902 under 15 years of age as the basis of the calculation. This number was 1,965 out of a total of 2,015 deaths. The table shows that in these differently constituted populations the crude or general death rate would vary more than 50 per cent., while the actual mortality was the same.

City.	Date of census.	Population by census.	Population under 15 years, by census.	Per cent., under 15 years.	Estimated population, 1902.	Estimated number under 15, 1902.	New York's diphtheria rate under 15.	Calculated diphtheria deaths under 15, according to New York rate for 1902.	Corresponding general death rate from diphtheria, 1902.	Actual diphtheria death rate to serve as comparison.
Berlin	1900	1,888,577	486,057	25.74	1,909,731	491,590	1.75	860	.45	.28
London.....	1901	4,536,541	1,357,874	29.93	4,579,110	1,370,600	1.75	2,398	.52	.30
Munich	1895	407,307	98,678	24.47	509,000	124,570	1.75	218	.43	.51
Paris	1901	2,657,335	501,512	18.87	2,695,065	508,750	1.75	890	.33	.24
Vienna	1900	1,674,957	455,790	27.22	1,701,989	471,200	1.75	823	.48	.20
New York.....	1900	3,437,202	1,053,298	30.64	3,665,735	1,123,400	1.75	1,965	.53	.53

EXTENT TO WHICH ANTITOXIN IS USED IN NEW YORK CITY.

In order to ascertain this for New York City at the present time, we have investigated all the cases of diphtheria occurring in New York during a period of about three weeks in November and December, 1905.

The total number of cases reported to the Department during this time was 385. In all but sixty of these reports stated that antitoxin had been administered.

In nine of these, however, antitoxin was subsequently administered, leaving 51 cases which did not receive antitoxin.

Of these 51 cases, six were thrown out by bacteriological examination as not being Klebs-Loeffler diphtheria, leaving only 45 cases of true diphtheria in which no antitoxin was given.

Of these cases, three died, as follows: 2 severe cases, no physician in attendance; 1 laryngeal case, died late in the disease the day after a physician was first summoned.

With the exception of the fatal cases just mentioned, and one case reported as being "moderately severe," all of these "no-antitoxin" cases were mild, and this fact in almost all the instances determined the decision of the physician in not administering antitoxin.

This would show, therefore, that about $\frac{1}{8}$ of the cases reported as being probably diphtheria, do not receive antitoxin, and that this remedy is used in almost all the severe cases attended by physicians.

VIABILITY OF KLEBS-LOEFFLER ORGANISMS FROM DRIED PSEUDO-MEMBRANE OF A RAPIDLY FATAL CASE.

BY ANNA I. VON SHOLLY, M. D.,

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On August 21, 1905, pseudo-membrane was obtained from a rapidly fatal case of diphtheria in which at the autopsy the pseudo-membrane was found to extend down from the trachea into the smallest visible bronchi. Several bits of this membrane about 1 c. c. in diameter, and at the same time the finely powdered membrane, were dried on glass in diffuse daylight.

At successive intervals emulsions of each were made in 1 c. c. of sterile water. These emulsions were about the opacity of a 24-hour typhoid culture in broth. From these, blood agar plates and Loeffler serum tubes (as controls) were inoculated. Unfortunately, too long a time was allowed to pass after the third inoculation, so that the period of the exact death of the organisms lies in rather wide limits between the middle of the third week and the fifth week. This time was allowed to elapse because we feared that the material would be used up too soon. The somewhat greater rapidity of the death of the bacilli in the finely divided membrane, as one would expect, is clearly shown. The Loeffler serum tubes also showed this very prettily. Whereas on the fourth and eighth days the dried intact membranes give a diffuse confluent growth, the powdered membranes gave discreet colonies. Only on the 17th day did the colonies from the intact membrane become discreet.

The results were as follows:

August 21, 1905.	August 25, 1905, dried 4 days.	August 29, 1905, dried 8 days.	September 7, 1905, dried 17 days.	September 26, 1905, dried 36 days.
Dried intact mem- brane.....	60,000,000 colonies to 1 c.c.	24,000,000 colonies to 1 c.c.	15,000,000 colonies to 1 c.c.	} No growth.
Dried powdered membrane.....	39,000,000 colonies to 1 c.c.	9,000,000 colonies to 1 c.c.	3,000,000 colonies to 1 c.c.	

On September 29, 1905, (39 days dried) an emulsion twice as thick was tested with no resulting growth, and serum broth tested the following day with large amounts of membrane was also negative.

VIRULENCE OF DIPHTHERIA-LIKE BACILLI ISOLATED FROM NORMAL THROATS OF CHILDREN.

BY ANNA I. VON SHOLLY, M. D.,

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The following is a preliminary report on the relative virulence of diphtheria-like organisms isolated from normal throats during the six months August 30, 1905, to February 13, 1906. By diphtheria-like bacilli we mean all organisms which show the typical morphological appearance characteristic of the Klebs-Loeffler bacillus. The so-called "Pseudo-Diphtheria" or Hoffman's bacillus is excluded.

In 1894, Park and Beebe examined culturally 330 throats of normal individuals and found diphtheria-like organisms in 32 cases or 9.7 per cent. of those examined. Of these 8 or 2.4 per cent. were virulent to guinea pigs and 24 or 7.3 per cent. non-virulent. Since then, what with the more careful isolation of the cases and the more general use of antitoxin, etc., the virulence of intensity of diphtheria in this section of the country as shown by the mortality-curve, has steadily declined. As this year, 1905-06, has been an especially mild diphtheria year it was thought that a comparison of present conditions with those obtained in 1894 might be profitable.

The cases examined up to the middle of January were obtained from Dr. Southworth's clinic at the dispensary of the Babies' Hospital, Fifty-fifth street and Lexington avenue, while the large majority of those examined since then have come from the children's clinic at the New York Infirmary for Women and Children, Fifteenth street and Lexington place, through the courtesy of Dr. Daniel. I mention this as one of the factors usually considered as bearing on the results, inasmuch as the patients of the latter clinic are a much poorer class, chiefly foreign—Russian and Italian—and consequently are much more poorly nourished and live under much less hygienic conditions. All throats which appeared normal to the eye were swabbed, irrespective of the disease from which the child was suffering, with the exception only of those cases where there was a nasal discharge suggestive of mild nasal diphtheria or a laryngeal disturbance. The larger number of the younger cases under

a year were purely feeding cases, while the older ones suffered variously from rachitis, digestive disturbances, anemia, diarrhoeas, enlarged tonsils and adenoids, etc., and a few of pertussis and mumps.

The following table gives the number of cases examined according to season together with the total for the six months and the results obtained.

TABLE I.

1905-6.	Number of Cases examined.	Diphtheria-like bacilli isolated from	Virulent K. L. bacilli isolated from	Non-virulent K. L.-like bacilli isolated from.	Pseudo-diphtheria bacilli in	Xeroses-like bacilli in
August 30-31.....	21
September.....	30	1 (3%)	..	1	1 (3%)	..
October.....	31
November	10	6 (6%)	..
December	27	4 (15%)	..	4	4 (15%)	..
January	69	5 (7.2%)	2 (3%)	3	35 (50%)	2
February 1-13.....	42	2 (4.7%)	1	1	15 (35%)	..
Total.....	230	12 (5.2%)	3 (1.3%)	9 (3.9%)	61 (26.5%)	2
Park and Beebe, 1894.....	330	32 (9.7%)	8 (2.4%)	24 (7.3%)

In all cases where the pseudo-diphtheria bacillus was found, where there was any doubt as to its identity, cultural tests and animal inoculations were used to establish it. Otherwise it was simply plated out and isolated.

The virulence test was made by inoculation of 200 to 300 gm. guinea pigs with 48-hour cultures in ascitic broth of the strain isolated. At the outset, 1 c.c. and $\frac{1}{2}$ c.c. injections were made subcutaneously and controlled by antitoxin. If the pigs died within the 4 days, still smaller doses up to 1-10 c.c. were given, each being always controlled by antitoxin. When the animal did not succumb to the initial doses, 10 c.c. doses were given.

Of the three virulent strains 1-10 c.c. killed in 24 hours in one case, and in two cases $\frac{1}{2}$ c.c. in 48 hours, the control pigs living.

Of the 8 non-virulent cases, 10 c.c. doses had no effect at all on the seven pigs. One case just recently isolated is still under test. Two organisms isolated, which were morphologically like Klebs-Loeffler bacillus of the barred type, behaved somewhat differently culturally from the ordinary diphtheria bacillus and were non-virulent. In 10 c.c. doses in one case both the non-antitoxin as well as the control antitoxin pig died and in the other the control pig alone died. These organisms were identified as belonging to the so-called xerosis group.

The ages of the children examined come under wide limits, varying from 1 month to 15 years, almost one half, however, falling under 3 years. Table II. gives the ages with the number examined and the number from which Klebs-Loeffler like organisms were isolated and their virulence:

TABLE II.

Age.	Number of Cases.	Diphtheria-like bacilli in.	Virulent K. L.	Pseudo-diphtheria.
4 weeks to 1 year.....	51	1	..	13
1 to 2 years.....	30	1	1	4
2 to 3 years.....	20	1	..	7
3 to 4 years.....	26	2	..	10
4 to 5 years.....	13	4
5 to 6 years.....	19	2	1	6
6 to 7 years.....	10	1
7 to 8 years.....	10	2
8 to 9 years.....	9	1	1	3
9 to 10 years.....	6	1	..	3
10 to 15 years.....	23	2	..	7
Exact age not known.....	13	1
Total.....	230	12	3	61

A STUDY OF PNEUMOCOCCI: A COMPARISON BETWEEN
THE PNEUMOCOCCI FOUND IN THE THROAT SE-
CRETIONS OF HEALTHY PERSONS LIVING IN
BOTH CITY AND COUNTRY AND THOSE
OBTAINED FROM PNEUMONIC EXU-
DATES AND DISEASED MUCOUS
MEMBRANES.

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The investigations carried on in the Research Laboratory were planned after consultation with the members of the Commission for the Investigation of Acute Respiratory Diseases, of the Health Department of The City of New York, but were otherwise entirely independent of that body. The study of the agglutination characteristics was undertaken by Dr. K. R. Collins, whose report follows this. The investigations are still being carried on and these preliminary reports are made at the suggestion of the Commission, so that all the workers in carrying on further studies might receive help from work already done.

PLAN OF INVESTIGATIONS.

In this study the following points have been considered:

I.—The presence of pneumococci (1) in normal sputum, (2) in pneumonic sputum and autopsy material, (3) in the sputum or exudates from pathogenic cases other than pneumonias.

II.—The comparison of the strains obtained from the different sources in the following particulars: (1) morphological and cultural characteristics, (2) virulence, (3) serum reactions.

The scheme of the work, which was carried out more or less fully, is tabulated as follows:

TABLE II.

GENEALOGICAL RECORD.

No. of Case.

1002 = Animal series. (Description of original material.)

- 1st rabbit, inoc. with 3 c.c.**
 - 3122** = Inulin-serum medium from 1st rabbit (description).
 - 2122** = Streak blood-agar plate from 1st rabbit (description).
 - 1122 = serum-broth culture from 1st rabbit (description).**
 - 3d rabbit, 0.1 c.c. inoc.**
 - 3332** Inulin-serum medium from 3d rabbit (description).
 - 2322** Streak blood-agar plate from 3d rabbit (description).
 - 1322** Serum-broth medium from 3d rabbit (description).
 - 2d rabbit, 4 c.c. inoc.**
 - 3222** Inulin-serum medium from 2d rabbit (description).
 - 2222** Streak blood-agar plate from 2d rabbit (description).
 - 1222** Serum-broth medium from 2d rabbit (description).
- 1st mouse, inoc. with 0.5 c.c.**
 - 3112**

Meaning of the numbers is the same as the above, except 1 instead of 2 in units place indicates that mouse has been used instead of rabbit, and consequently the numbers in hundreds place, indicating the doses, have a different meaning.
 - 2112**
 - 1112**
 - 4th mouse, 0.0001 c.c. inoc.**
 - 3412**
 - 2412**
 - 1412**
 - 3d mouse, 0.01 c.c. inoc.**
 - 3312**
 - 2312**
 - 1312**
 - 2d mouse, 0.5 c.c. inoc.**
 - 3212**
 - 2212**
 - 1212**

Name and short history of case.

Keeping of Records—Geneological tables of each strain have been kept, a modification of the Dewey Library System of numbers being used to indicate the cultures. Thus by referring to these tables one is able quickly to get the principal points in the history of a particular culture from the time of its isolation.

Numbers of four denominations have been used, the units place indicating the series, the tens place the animal used for inoculations, the hundreds place the dose received, and the thousands place the medium employed. Underneath this number the number of culture generations is placed in parentheses. Table II. is one such geneological table.

In addition to these tables, comment sheets on each strain and tables of comparative morphology and cultural peculiarities have been kept.

THE PRESENCE OF PNEUMOCOCCI.

Two hundred cases have been examined for the presence of the pneumococcus. In the great majority of cases two methods—(a) animal inoculation of mass cultures and (b) stroke blood-agar plates, as shown in Table I.—have been employed in attempts at isolation; in the other cases only one of these two methods has been used. Table III. shows the grouping of the cases and the number in which typical and atypical pneumococci have been found.

From this table we see that typical pneumococci have been obtained in a large percentage of normal cases in both city and country. A few pneumococci may have been missed because of occasional contaminations or overgrown cultures or the employment of large rabbits or some other cause. In the majority of cases where no pneumococci were found streptococci were isolated. From a series of autopsies on cases of broncho-pneumonia at the Willard Parker Hospital, and from a series of pertussis sputa from the Foundling Hospital, large numbers of influenza-like organisms were found with smaller numbers of streptococci and occasionally with a few pneumococci. It was very difficult to get rid of these influenza-like organisms, as great numbers passed through the animal inoculated with mass-cultures, and in the plates and serum-broth tubes they grew abundantly in close association with the

pneumococcus. Repeated platings generally had to be made before a pure culture of the pneumococcus could be obtained in these cases.

TABLE III.

Showing Number of Cases Studied and Number in Which Pneumococci Were Found.

Groups.	Subdivisions.	Number of Cases.	Pneumococci not Isolated.	Pneumococci Isolated.	
				Atypical.	Typical.
	Research Laboratory.....	3	3
	Bellevue Students.....	10	2	1	7
	Saranac Lake.....	28	15	3	11
	Sea Breeze.....	5	3	.	2
	Tarrytown	1	1
	Foundling Hospital.....	9	2	..	7
	Briarcliff.....	6	3	..	3
	Hyde Park.....	4	..	4	..
	Millbrook.....	7	..	2	5
	Newburgh.....	5	..	1	4
	Babies' Hospital.....	2	2
Pneumonia.	Lobar-	53	4	5	45
	Broncho-	21	5	2	14
Colds.	15	5	..	10
Miscellaneous.	Measles	3	1	..	2
	Scarlet-fever	5	3	..	2
	Tuberculosis	5	3	..	2
	Pertussis.....	5	4	..	1
	Influenza.....	2	2
	Pleurisy.....	1	1
	Typhoid.....	1	1
	Mastoiditis.....	1	1
	Synovitis.....	3	1	..	2
	Meningitis	1	1
	Œdema of Lungs.....	1	1
	Empyæma.....	3	1	..	2

COMPARISON OF STRAINS.

Morphological and Cultural Characteristics—We have divided the pure cultures of pneumococci obtained into two broad groups according to their morphological and cultural characteristics. The first group is composed of typical pneumococci and the second of atypical ones.

By typical pneumococci we mean cocci which (1) under certain more or less constant cultural conditions occur principally in slightly elongated and pointed twos with broader ends apposed, (2) under similar or other cultural conditions form capsules, (3) when grown in inulin-serum¹ medium produce coagulation, and (4) when grown in poured blood-agar plates produce a distinct green color in and about colonies.

By atypical pneumococci we mean (1) cocci which morphologically and culturally resemble more or less closely the pneumococcus except in their growth in the Hiss inulin medium, which they do not coagulate; (2) cocci which are like streptococci morphologically, but which produce coagulation of the Hiss inulin medium.

Referring to Table III. we see that a larger number of atypical strains have been obtained from normal cases, in all of which only sputum was studied, than from pathogenic. This may be due simply to the fact that so many more typical pneumococci were present in the majority of pathogenic cases studied that the atypical ones may have been missed in some of these cases. Atypical pneumococci of the first group, *i. e.*, those which do not coagulate inulin-serum medium, have been found as the majority of colonies and as the only pneumococcus-like

¹ The inulin used in the course of the present work in making up Hiss' medium (*Jour. of Exper. Med.*, 1905, vii., 317) was prepared in this laboratory by R. B. Gibson, for at the time we were unable to obtain it from commercial sources. The method employed in the obtaining of this substance follows: Dandelion (*taraxacum*) roots were soaked in cold tap water until soft, and if coarsely ground the roots were then run through an ordinary hash machine. The material was transferred to a gauze bag, and was washed thoroughly in running cold tap water to remove a portion of the soluble impurities and the finer solid particles which would interfere with subsequent filtration. The washed roots were extracted in boiling water, strained, and filtered. A second immediate extraction followed. The filtrates were united and evaporated over a Bunsen burner to a thin syrup. Alcohol (10-15%) was added and the mixture was cooled to 0° or below. The inulin separating out on standing was thoroughly washed by decantation with cold alcohol (10-20%) and then with 95% alcohol. It was filtered into a suction funnel washed on the filter with hot alcohol sucked dry, and finally spread on filter paper in a warm place to remove the alcohol still remaining. The resulting product can be obtained as a fine white powder which gives the ordinary reactions of inulin; solutions of this production do not reduce Fehling's solution. The yield from five pounds of the tubers was about three hundred grams.

organism in the sputum from two cases of pneumonia, and have been accompanied by typical pneumococci in the sputum from three other cases. So far they have not been found in autopsies following pneumonia. In one autopsy case and in one broncho-pneumonic sputum large numbers of cocci of the second group of atypical pneumococci were found.

It is interesting to note that, when some of the non-coagulating cultures were studied more minutely, various colonies being fished and the resulting cultures being tested for their ability to coagulate serum-inulin media, in the case of one culture one colony out of six produced late coagulation. From this coagulating colony, however, no further colonies were obtained producing coagulation. Among the typical pneumococci all strains vary somewhat with regard to their power to coagulate the Hiss medium, a few producing very late coagulation. When individual colonies were fished from some of these latter strains, it was found that there was a wide variation in the time required for coagulation, an occasional one not coagulating at all. It seems, from these observations, that the non-coagulating, more or less morphologically typical pneumococci are closely related to the typical late coagulators. One of these atypical strains showed typical capsules in the heart's blood of animals and the other showed occasional small capsules.

All of the typical and atypical strains, as well as many strains of streptococci, have produced a green color in and about colonies in poured blood-agar plates, while other streptococci have produced large areas of hæmolysis about colonies and no green color. These results agree in part with those of Schottmüller (*Munchener med. Woch.*, 1903, p. 849), and E. Fränkel (*Munchener med. Woch.*, 1905, p. 548), who divide streptococci into three groups according to their behavior in blood-agar plates: *Streptococcus pyogenes* producing much hæmolysis, *streptococcus virideus* producing green color, and *streptococcus mucosus* producing mucous-like material as well as green color. These results differ from those of Rosenow (*Journal of Infectious Diseases*, 1904, I, 280) who states that no streptococcus tried by him produced green color, while all pneumococci did, and he therefore recommends this test in differentiating the two species. From the sputa of a number of

cases of broncho-pneumonia we tried to isolate the pneumococcus by this method, making poured blood-agar plates from different dilutions of the sputum and fishing from the green colonies, and at the same time we used the method of animal inoculation by mass-cultures. In every case by the first method only streptococcus-like organisms were obtained, while by the second typical pneumococci were isolated.

All of the strains of typical pneumococci studied by us may be divided into several distinct morphologic varieties. We call them varieties, because while each strain shows a wide limit of fluctuating variability, certain strains have similar predominating constant characteristics. These varieties are:

1. Small cocci occurring under most cultural conditions in twos and producing small capsules.
2. Large cocci occurring readily in short and medium-length chains and producing large capsules.
3. The so-called streptococcus mucosus.

The first two varieties are less distinctly bounded than the last which forms a definite morphologic variety. This variety, which has been mentioned only a few times in literature (Schottmüller, *Munchener med. Woch.*, 1903; L. Buerger, *Medical News*, 1904, p. 1117; E. Fränkel, *Munchener med. Woch.*, No. 12, 1905; L. Heim, *Zeit. für Hyg.*, 1905, I., 139), has been classed as a streptococcus, under the name of streptococcus mucosus by Schottmüller, and streptococcus mucosus capsulatus by others. It has been isolated by us from eight cases of pneumonia, from two cases of cold, and from two normal individuals, and has been seen in mixed cultures in a number of other cases. Three of the cases of pneumonia were early autopsy cases. In two of these the organism occurred pure and in large numbers (two hundred colonies were fished in one case and the resulting cultures were all similar); in the third case it was accompanied by a smaller number of the first variety of typical pneumococci. In one pneumonic sputum and in one normal individual the first variety of typical pneumococci also accompanied it, while in all the other cases it was the only pneumococcus-like organism isolated. It has thus been found by us more frequently in cases of pneu-

monia than in other cases. We have classed it among the typical pneumococci for the following reasons:

1. On serum-free culture media after the first two or three culture generations it produces no mucous-like material and shows no capsule or chain formation, but appears like a typical pneumococcus.

2. It readily coagulates the Hiss inulin medium.

3. It shows very distinct capsules in serum media and in the blood of inoculated animals.

4. It has been found pure and in large numbers in two cases of typical lobar pneumonia.

5. The results obtained from absorption experiments (see the Collins report) indicate a close relationship between it and certain typical pneumococci of the second variety, while no relationship is shown between it and the strains of typical streptococci tested.

It has been classed with the streptococci heretofore because of its ability readily to produce rounded forms and short chains. According to our descriptions of typical and atypical pneumococci it might be classed by many with the latter, but considering its ability under certain conditions to show typical pneumococcus forms we prefer to class it with the former, and make it a distinct variety. With regard to nomenclature, it should be called, according to the classification followed, *Streptococcus lanceolatus*, var. *mucosus* (the classification of Lehmann and Neumann, which we prefer), or *Diplococcus lanceolatus*, var. *mucosus* (the classification of other authors); we have given it the trivial name, *pneumococcus mucosus*.

By referring to the section on serum reactions below and to Dr. Collins's report on the agglutination of the pneumococcus, it will be found that all the strains of this variety isolated by us show a specific similarity in these reactions.

A certain number of cultures from both normal and abnormal cases, which showed the characteristics of typical pneumococci immediately after isolation, have later dropped some of these characteristics and become more like streptococci. They appear principally in chains and no longer coagulate the inulin-serum medium. Whether some of these cultures were mixed in the beginning with a streptococcus-like organism

growing in intimate connection with the pneumococcus, as the influenza bacillus does, and finally outgrowing it, or whether they are all mutating varieties, is still a question. With such a mass of cultures it was impossible to follow each closely, to make plates, and to study colonies of each new culture generation, but, judging from the few apparently changing strains which have been more minutely studied, it would seem as if some of these cultures were really changing by mutation. None of them have become permanently typical streptococci—that is, they show more or less irregularity in chain production, sometimes produce elongated and pointed twos and always green color in blood-agar plates, but they seem gradually to lose their power to coagulate inulin-serum medium. These observations in regard to mutating varieties indicate a close relationship between certain pneumococci and streptococci, a relationship which previous investigators have noted.

All strains of pneumococci tried coagulated, usually within forty-eight hours, serum media containing dextrose, lactose, or saccharose, as do also certain strains of streptococci. With mannit different strains of pneumococci act differently. Out of one hundred strains tested, twenty-nine did not coagulate mannit-serum medium after fourteen days. Among the seventy-one coagulators, sixteen coagulated in twenty-four hours, seventeen in forty-eight hours, one on the third day, five on the fifth day, and the rest between the fifth and fourteenth days. With the exception of the mucosus variety, there seems to be no relation between this coagulation and the varieties or groups of pneumococci. All of the pneumococcus mucosus strains tested coagulated the mannit medium within two days. Certain atypical strains which did not coagulate the inulin readily coagulated the mannit medium, while the few definite streptococci tried did not coagulate either. The plate growths from these non-coagulating cultures all showed practically as many colonies as those from the coagulating ones.

Virulence—The virulence of the different strains of pneumococci for lower animals depends in great measure upon the method of isolation used. If the plate method be employed, fishing individual colonies, the majority of pure cultures obtained will be distinctly less virulent than those isolated by the mass-culture method. The mass-culture method consists in inoculating a mass of sputum or material to be tested into

serum-broth (previously tested for ability to give abundant growth of pneumococci), placing at 36 degrees C. for twenty-four hours, and inoculating a certain amount of the resulting culture subcutaneously into the animal chosen. The culture isolated from the heart's blood of the animal at autopsy is then tested for virulence in the same species of animal.

We have used both rabbits and white mice for the inoculations, but in the great majority of cases the former animals only. Young rabbits, weighing from 800 to 1000 grams, and young adult mice have been chosen.

By testing the virulence of strains isolated by the mass-culture method, it has been shown that the percentage of virulent strains of pneumococci isolated from cases of pneumonia is higher than the percentage of those isolated from normal cases (see Table IV.).

TABLE IV.
Percentage of Virulent Strains.

Amount Inoculated.	Pneumonia Cases.	Healthy Individuals.
4.0 Cubic centimeters.....	87 Per Cent.	69 Per Cent.
0.1 Cubic centimeter.....	51 Per Cent.	31 Per Cent.

Most of the strains isolated from the cases of broncho-pneumonia which are included with the cases of pneumonia are not very virulent, while most of the strains from the colds which have not been noted here are virulent. Among the normal individuals the largest percentage of virulent pneumococci came from the Foundling Hospital children, the next from the Bellevue students, the next from the country around New York, and the smallest from Saranac Lake. Too much weight should not be attached to this summary, because of the comparatively small number of cases examined.

Normal No. 40 in contact with pneumonia No. 36 were of equally extreme virulence for both mice and rabbits. All of the pneumococcus mucosus strains tested have been with one exception extremely virulent for mice and decidedly less so for rabbits.

Retention of Virulence—Grown on artificial media, all of the virulent strains are losing their virulence although those transplanted on media containing blood from the species of animal used for the testing remained more virulent longer, for that species than for the other species of test animal chosen. No. 36, however, one of the most virulent strains tested, remained virulent for a long time for both rabbits and mice when grown on rabbit blood-agar. It seems now gradually to be losing its virulence for both animals. When grown in Bolduan's calcium-broth medium (see page 137), cultures of pneumococcus remain alive and retain their virulence as long as when grown in serum-broth according to the few tests made; therefore, as this medium generally allows an abundant growth, it is an excellent one to use when for any reason the use of serum is undesirable.

Agglutination reactions are described in a separate report by Dr. Collins.

SERUM REACTIONS.

Specific Protective Substances—According to Neufeld and Rimpau (*Deutsch med. Woch.*, 1904, p. 1458), the serum of rabbits inoculated with pneumococcus cultures becomes speedily protective for white mice. They claim to have obtained after the second inoculation of large doses of pneumococcus bodies, the first killed by heat and the second living, a serum which was highly protective for mice. They claim that this serum has no lytic properties for the pneumococcus, but that the specific protective substance is a bacteriotropic substance uniting with the bacteria and preparing them for ingestion by the leucocytes, and that when this serum is added to a mixture of bacteria and normal leucocytes in vitro more phagocytosis is produced than when normal serum is used. So far Neufeld's bacteriotropic substance agrees with Wright's (*Proceedings of the Royal Soc. of London*, 1903, LXXII., 337) opsonic substance, except that Neufeld claims that his substance is not destroyed by low heat, while Wright says that his is. Therefore Neufeld states that his bacteriotropic substance is not the same as Wright's opsonic substance.

Very little as yet has been done by us in attempting to raise in animals specific protective substances for the pneumococcus or in studying the properties of such substances. In the beginning we followed Neufeld's method, inoculating large doses of surface cultures of pneumococci into

rabbits. The first cultures were subjected to from 60 degrees to 65 degrees C. for from fifteen to thirty minutes and the subsequent cultures were living. There is no doubt that a preliminary large dose of a dead culture could be followed by a larger dose of a living culture without causing death than if a small preliminary dose had been used, but the serum of such rabbits showed no protective action in mice with any of the strains of pneumococcus tested. Only a few tests were made, however, so no definite conclusion can be drawn. The phagocytic power in vitro seemed to be slightly increased for some of the strains, each by its own serum.

It was found that the opsonic power of normal rabbit, sheep, and especially of normal horse serum is very great for some strains of pneumococci, less so for others, and very slight for others. All of the strains of pneumococcus mucosus tested belong to this last group. Since rabbits proved unsatisfactory, it was decided to experiment with sheep. Two sheep were chosen, one of which was inoculated with one of the first variety of pneumococcus and the other with a strain of pneumococcus mucosus. The sheep have received eleven inoculations and have been bled twelve times, as is shown by the following table:

TABLE V.
Inoculations and Bleedings of Sheep.

Amount Inoculated.	Date of Inoculation.	Date of Bleeding.
20 c.c. of 24-hr. broth cult. centrifugalized and exposed to 60° C. for 20 min.	March 3
27 c.c. of 24-hr. broth cult. centrifugalized and exposed to 60° C. for 20 min.	" 10	March 15
28 c.c. of 24-hr. broth cult. centrifugalized and exposed to 60° C. for 10 min.	" 17	" 24
32 c.c. of 24-hr. calcium-broth cult. centrifugalized.	" 29	April 2
40 " centrifugalized	April 8
50 " centrifugalized	" 19	April 27
60 " centrifugalized	" 28	May 4
70 " centrifugalized	May 5	" 10
80 " 30 c.c. centrif.	" 15	" 24
85 " 30 c.c., centrif.	" 27	June 5
50 + 10 slant blood-	June 8	" 15
	" 21

The serum from each bleeding was tested in vitro for its opsonic or bacteriotropic power on a number of strains of pneumococci, and from a few of the bleedings it was tested in addition for its protective power in white mice. In testing the opsonic power of the serum in vitro the following technic was used: To 0.5 c.c. of serum, undiluted or diluted, in a short wide test tube, were added 0.5 c.c. of a thick suspension of washed normal leucocytes and 0.5 c.c. of the dilution of bacteria. The washings and dilutions were made with 0.8 per cent. of sodium chloride solution. The mixtures were kept at 36 degrees C. and smears made at stated times. The leucocytes almost immediately form a thin layer about the sides and bottom of the test tube and a well spread smear containing large numbers of leucocytes is made by scraping from this layer with a flatly coiled platinum loop and spreading quickly on a clean glass slide. The smears were fixed in methyl alcohol and stained with eosin and methylene blue. Normal leucocytes from rabbits, guinea-pigs, sheep, and horses have been used, and so far our results have agreed with those of all other observers in regard to the indifferent action of leucocytes from different species of animals. According to our experiments, some species of leucocytes need more careful washing than others, probably because of the greater opsonic power of the corresponding normal serum. For example, horse leucocytes must be most carefully washed in order to keep the controls from showing phagocytosis. We have used horse leucocytes for many of the experiments because of our ability to obtain them easily and quickly in large quantities. The horse is bled just before the leucocytes are to be used and the blood is collected aseptically in flasks, with one tenth its volume of a 10 per cent. solution of sodium citrate in normal salt solution. After mixing, the blood is allowed to stand, and within ten minutes the red blood cells have settled, leaving the plasma, containing many leucocytes, above. This is drawn off, centrifugalized, and the leucocytes are washed carefully four times; each time fresh sterile plugs are used for the tubes. In this way it is easy to obtain a large amount of a very thick suspension of actively motile polynuclear leucocytes. Of such a suspension 0.5 c.c. added to the mixture of 0.5 c.c. each of 0.8 per cent. salt solution and the required dilution of bacteria has been used as one control, and a similar

mixture with normal serum in the place of the 0.8 per cent. salt solution as another.

The dilutions of the bacteria were prepared as follows: A twenty-four-hour calcium-broth culture made from a twenty-four-hour blood agar-slant culture of the stock culture (the blood-agar was made from rabbit blood and kept in the thermostat at 36 degrees C. for two days before using) was centrifugalized and enough 0.8 per cent. salt solution added to the bacteria to make a suspension of about 2,000,000,000 bacteria to the cubic centimeter.

In estimating the phagocytic action by this method, it has been found that a large number of polynuclear leucocytes must be counted, as phagocytosis seems to occur irregularly, a group of polynuclear leucocytes each one loaded with bacteria filling one field, and a group containing no organisms the next.

The mixtures were examined in the beginning, at the end of one quarter, one half, two, three, five, and twenty-four hours. It was found that the difference between the serum controls or heated serum and the specific serum was more marked after fifteen to thirty minutes than at the height of phagocytosis, which occurred in from two to three hours. At the latter time the differences, if any, were very slight. The specific serum thus seemed to allow the phagocytosis to occur more quickly.

The difference between the opsonic power in vitro of the normal serum and the specific serum, however, has so far been slight. This slight increase of opsonic power of the specific serum was apparent after the second bleeding and continued up to and including the eighth bleeding, but the serum from the next two bleedings (ninth and tenth) showed no definite difference in phagocytosis between normal serum controls and specific serum. The serum from the ninth bleeding, however, showed a protective power for mice similar to that of the serum from the eighth bleeding, and the serum from the tenth bleeding prolonged life. As the control animals in these experiments all died, the absolute protective power of these sera is not known. From these data all that can be said is that while the phagocytic power in vitro of a certain specific serum seemed no greater than that of the control serum, yet the former possessed marked protective power in mice. One of the

heterologous strains (Pn. 4) showed clumping and marked phagocytosis with Sheep Serum II. (inoculated with *Pneumococcus mucosus*), while with Sheep Serum I. (inoculated with Pn. 36) it showed no clumping and less phagocytosis, and yet mice were protected from it by this latter serum. All of the *pneumococcus mucosus* strains showed very slight phagocytosis in any serum, and yet with Sheep Serum II. mice were protected from all of the strains with but one exception.

It seems from these observations that the degree of phagocytosis in vitro with some sera at least is not an indication of the degree of protective power in mice.

In regard to the influence of heat upon the phagocytic power of these sera, the following results have been obtained: 60 degrees C. for a half hour has slight deleterious effect, 65 degrees for twenty minutes has more, and 60 degrees for one hour has a marked effect.

Poured blood-agar plates after two hours at 36 degrees C. show a decrease in the number of colonies with all the strains which agglutinated, but the decrease is no greater than could probably be accounted for by the agglutination.

SUMMARY AND CONCLUSIONS.

1. Typical pneumococci were present during the winter months in the throat secretions of a large percentage of healthy individuals in city and country.

2. A higher percentage of atypical strains of pneumococci have been obtained from healthy persons than from those suffering from pneumonia. In the latter cases the atypical strains may have been overlooked, because of the larger number of typical pneumococci present. Many of the atypical strains seem to be closely related to the streptococci.

3. The so-called *streptococcus mucosus* Schottmüller, which has hitherto been classed with the distinct streptococci, is placed as a definite variety among the pneumococci, and it is recommended that the name be changed to *streptococcus lanceolatus*, var. *mucosus*.

4. A lower percentage of strains of pneumococci virulent for rabbits in the doses used has been obtained from normal cases by rabbit inoculations of mass cultures than from cases of pneumonia by the same method.

5. Since the virulence of pneumococci may be rapidly increased for a susceptible species of experimental animal by successive passage, and since pneumococci obtained from most pneumonias are more virulent for experimental animals than are those obtained from healthy individuals, therefore the virulence of pneumococci from cases of human infection is probably increased for human beings; hence cases of pneumonia should be considered to a certain degree as contagious and, since the virulence of the pneumococcus may be quickly increased and since the organism is very prevalent in normal sputum, all possible measures should be taken to restrict public expectoration.

6. By repeated inoculations into sheep of a pneumococcus strain, a specific protective power of this serum for mice is developed against the homologous strain and against certain other strains, one morphological variety (*streptococcus lanceolatus*, var. *mucosus*) being thus clearly differentiated from other strains.

7. Coincident with this production of protective power, a slight specific increase of the sheep serum in phagocytic power in vitro has been observed with some strains of pneumococci, all strains of *streptococcus lanceolatus*, var. *mucosus*, acting similarly with the serum produced by the inoculation of one strain; the strains of some other varieties, however, have shown no definite relationship between the phagocytic power and the protective power of the serum.

A STUDY OF THE PNEUMOCOCCUS DURING THE SUMMER OF 1905.

BY M. ALICE ASSERSON, M. D.,

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During the summer of 1905, the study concerning the presence of pneumococci, especially in normal individuals, was continued, because it was thought that the influence of season might be shown to be considerable. It was felt to be unnecessary to duplicate the entire number of places previously studied, but the results obtained may very fairly be compared with those during the winter of 1904-5 (see page 95).

During the course of the work we isolated a number of pneumococci from normal throats at the Foundling Asylum, and we therefore decided to make a comparative study of the same cases after an interval of one month with a view to noting the persistence of the organism, and whether or not there was a change in virulence.

The following table will show that out of the six cases examined in July, five show the persistence of the organism one month later. The virulence, however, had slightly lessened in each case.

Normal Cases from Foundling Hospital—July 6 to August.

Number of Cases.	Pn. Isolated.		Not isolated.	Morphology.	Inulin Test.*	Virulence Test: Broth Culture: Dose from 4 c. c.-1/100,000 c. c. in ear vein of rabbit.
	Typical.	Atypical.				
N. 86.....	+	Elongated diplococci of medium size, occurring singly..	+24 hrs. {	—Rabbit 3 c. c. subcut. alive.
N. 87.....	+	Rather large elongated pointed diplococci showing capsules.....	+48 hrs. {	† 1/1,000 c. c. in 3 days.
N. 88.....	+	Lance-shaped diplococci occurring singly. No capsules present	+24 hrs. {	† 4 c. c. in 3 days.
N. 89.....	..	+	..	Lance-shaped diplococci occurring singly. No capsules present.....	+24 hrs. {	† 4 c. c. in 2 days.
N. 90.....	+	Medium-sized lance-shaped diplococci with capsules...	+24 hrs. {	† 1/10 c. c. in 2 days.
N. 91.....	+	Medium-sized lance-shaped diplococci with capsules...	+24 hrs. {	† 1/10,000 in 3 days.
N. 92 same as N. 86 Aug. 28 1 month later	+	..	Small lance-shaped diplococci singly and in short chains. No capsules.....	+48 hrs. {	—3 c.c. subcut. alive.
N. 93 same as N. 87 1 month later	+	Medium-lance shaped organisms (25) singly and in short chains, no capsule...	+24 hrs. {	† 1/10 c. c. in 24 hrs.
N. 94 same as N. 88 1 month later	+
N. 95 same as N. 89 1 month later	+	Small lance-shaped diplococci singly and in very short chains with several capsules	+24 hrs. {	† 4 c. c. in 3 days.

* The sign + in this column means coagulation of the medium.

Normal Cases from Foundling Hospital—July and August.

Number of Cases.	Pn.Isolated.		Not Isolated.	Morphology.	Inulin Test.*	Virulence Test: Broth Culture: Dose from 4 c. c.-1/1,000 c. c. in ear vein of rabbit.
	Typical.	Atypical.				
N. 96 same as N. 90, 1 month later	+	{ Medium elongated pointed diplococci singly showing some capsules..... }	+24 hrs. {	-3 c. c. subcu- taneously— alive.
N. 97 same as No. 91, 1 month later	+	{ Medium lance-shaped diplo- cocci singly and in a few short chains surrounded by capsules..... }	+24 hrs. {	† 1/1,000 c. c. in 3 days.
N. 98.....	+
N. 99.....	+	{ Elongated pointed lance- shaped 2's surrounded by small capsules..... }	+24 hrs. {	† 4 c. c. in 24 hrs.
N. 100.....	+	{ Elongated lance-shaped diplo- cocci of medium size; also spherical diplococci, surrounded by large cap- sules..... }	+ 3 days. {	† 1/10 c. c. in 24 hrs.
N. 101.....	+
N. 102.....	+
N. 103.....	+	{ Medium lance-shaped diplo- cocci showing no capsules. }	+ 2 days. {	-3 c. c. subcu- taneously— alive.

* The sign + in this column means coagulation of the medium.

Normal Cases from the Research Laboratory—September 7, 1905.

Number of Cases.	Pn.Isolated.		Nor Isolated.	Morphology.	Inulin Test.*	Virulence Test. Dose of Broth Culture.
	Typical.	Atypical.				
N. 104.....	+	{ Small lance-shaped diplococci surrounded by capsules.... }	+24 hrs. {	Not tested; could not get growth from plates or as- citic broth cultures.
N. 105.....	+	{ Small diplococci, ovoid in shape, surrounded by cap- sules..... }	+3 days. {	† Rabbit 4 c.c. in 2 days. † Mouse, 1/100,000 in 6 days.
N. 106.....	+	{ Small lance-shaped diplococci surrounded by capsules.... }	+24 hrs. {	-Rabbit did not die. † Mouse, 1/100,000 in 2 days.
N. 107.....	+	{ Small lance-shaped ovoid diplo- cocci singly and ecap- sulated..... }	+24 hrs. {	† Rabbit 4 c.c. in 4 days. † Mouse 1 c.c. in 24 hours.
N. 108.....	+	{ Small lance-shaped diplococci with slender capsules..... }	+24 hrs. {	-Rabbit with 4 c.c. did not die. † Mouse died with 1/100 c.c. in 24 hours.

* The sign + in this column means coagulation of the medium.

Normal Cases from Laboratory at Fifty-fifth Street—September 15, 1905

Number of Cases.	Pn. Isolated.		Not Isolated.	Morphology.	Inulin Test.*	Virulence Test.
	Typical.	Atypical.				
N. 109.....	..	+	..	Very small elongated pointed diplococci singly and in short chains. No capsules.	+3 days.	—Rabbit, 4c.c. did not die. —Mouse, 1/100 c.c. did not die.
N. 110.....	+	Medium-sized lance-shaped diplococci surrounded by large capsules.....	+24 hrs.	†Rabbit, 4c.c. died in 3 days. —Mouse, 1/100 c.c. did not die.
N. 111.....	+	Small lance-shaped diplococci..	+3 days.	—Rabbit, 4c.c. did not die. —Mouse, 1/100 c.c. did not die.
N. 112.....	+
N. 113.....	+	Small lance-shaped diplococci showing medium-sized capsules.....	+24 hrs.	—Rabbit, 4c.c. did not kill. —Mouse, 4c.c. did not kill.

* The sign + in this column means coagulation of the medium.

Bellevue Students—October 18, to November 1, 1905.

Number of Cases.	Pn. Isolated.		Not Isolated.	Morphology in Smears from Heart's Blood of Animals.	Inulin Test.*	Virulence Test.
	Typical.	Atypical.				
C. 22, same as N. 125, 14 days later.....	+	Large diplococci more ovoid than lance-shaped showing capsules.....	+4 days.	†Mouse, 1/100.000 in 3 days. —Rabbit, 3c.c. subcut.—did not die.
C. 23, same as N. 130, 17 days later.....	+	Small elongated pointed diplococci surrounded by small capsules.....	+24 hrs.	Mouse, ½c.c. subcut.—did not die. †Rabbit, 4c.c. in 2 days.
N. 124..	+	..	Small diplococci lance-shaped singly and in clumps of short chains.....	+24 hrs.	—Mouse, ½c. subcut.—did not die.
N. 125.....	+	Small diplococci, some elongated others more flattened. Small capsules present.....	+2 days.	†Mouse, 1/100 c.c. in 4 days. —Rabbit, 3c.c. subcut.—did die.
N. 126.....	+

* The sign + in this column means coagulation of the medium.

Number of Cases.	Pn.Isolated.		Not Isolated.	Morphology in Smears from Heart's Blood of Animals.	Inulin Test.*	Virulence Test.
	Typical.	Atypical.				
N. 127, sl. cold.....	..	+	..	Small diplococci, few elongated and pointed, others more spherical. Singly and in short chains.....	+2 wks.	- Mouse, 1/100c.c. - Rabbit, 3c.c. subcut. --did not die.
N. 128.....	..	+	..	Small sl. elongated diplococci also flattened diplococci singly and in short chains. No capsules.....	-	† Mouse, 1/100c.c. in 5 days. - Rabbit, 3c.c. subcut. --did not die.
N. 129.....	+
N. 130.....	+	Medium sized lance-shaped diplococci.....	+48 hrs.	- Mouse, 1/100c.c. - Rabbit, 3c.c. subcut. --did not die.
N. 131.....	+	Medium sized lance-shaped diplococci singly and in short chains with large capsules.....	+48 hrs.
N. 132.....	+
N. 134.....	+
N. 135.....	+	Small elongated and pointed diplococci showing large capsules.....	+6 days.	† Mouse, 100 c.c. in 2 days. † Rabbit, 4 c.c. in 6 days.
N. 136. Same as N. 131, 14 days later.....	+	Medium sized lance-shaped diplococci with large capsules.	+7 days	† Mouse, 1-100 c.c. in 2 days. - Rabbit, 3 c.c. subcut. --did not die.
N. 137. Same as N. 124, 14 days later.....	+	Large lance-shaped diplococci with capsules.....	- Did not grow inulin	† Mouse, 1-100,000 in 2 days. - Rabbit, 4 c.c. did not die.
N. 138. Same as N. 126, 14 days later.....	+
N. 139. Same as N. 128, 14 days later.....	+	Small lance-shaped diplococci singly and in long chains, with capsules.....	- Did not grow.	- Mouse, 1/2 c.c. subcut. did not die. † Rabbit, 4 c.c. in 18 days.
N. 140 (Sl. cold)....	+

* The sign + in this column means coagulation of the medium.

Cases from Quarantine Hospital—June 15, 1905.

Number of Cases.	Pn. Isolated.		Not Isolated.	Morphology.	Inulin Test.*	Virulence Test.**
	Typical.	Atypical.				
Q. 2.....	+	Elongated lance-shaped diplococci occurring singly and in short chains..... Ovoid and elongated diplococci occurring singly and in short chains surrounded by small capsules..... Medium sized lance-shaped diplococci occurring singly. No capsules.....
Q. 3.....	+		+ 24 hrs. {	4c.c. did not kill.
Q. 4.....	+		+ 24 hrs. {	4c.c. did not kill.
Q. 5.....	..	+	..		+ 24 hrs. {	4c.c. did not kill.

* The sign + in this column means coagulation of the medium.
** Virulence was tested with 24 hr. ascitic broth cultures.

The above table shows the results obtained from a few cases from the Quarantine Hospital. These patients were detained at the hospital on account of certain indefinite symptoms—not pneumonia. As they represented different foreign countries, and had been at sea for a period of several days, it was considered interesting to ascertain to what extent they harbored the pneumococcus. As may be seen from a study of this table, the pneumococcus was isolated from 3 out of 4 cases, but none of these were virulent.

All Normal Cases.	Number of Cases.	Pn. not Isolated.	Pneumococci Isolated.	
			Atypical.	Typical
Research Lab. (Sept. 7, 1905).....	5	5
Diagnostic Lab. (55th St.).....	5	1	1	3
Quarantine Station Immigrants.....	5	5
Bellevue Students.....	18	7	2	9
Foundling Hospital.....	18	4	3	11

The work during the winter months showed that the pneumococci from normal cases were virulent in 69 per cent. of the cases when 4 c.c. of a serum broth culture was injected into rabbits and in 31 per cent. when only 1 c.c. was employed.

In the above cases examined during the summer the results were as follows:

With a 4 c.c. dose..... 33 per cent.
With a 0.1 c.c. dose..... 7 “

A COMPARISON OF PNEUMOCOCCUS STRAINS IN RECENT AND ORIGINAL TESTS.

BY JANE L. BERRY, M. D.,

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The accompanying tables give the results of a recent study of some of the pneumococcus strains isolated during the past year, as compared with their morphology and reactions when first observed.

Very few strains are now found to be entirely typical morphologically. The majority show very small organisms and increased chain formation, and some are decidedly atypical in either serum broth or plate cultures, or in both. Unless extremely small, size of organisms has not been considered in the present division into characteristic and non-characteristic strains, this division being based upon the general morphological picture.

Of the 27 pneumonia cultures studied, 9 coagulated inulin not later than in the original tests, 7 of these fairly characteristic morphologically, 2 not characteristic; 6 coagulated later than in original tests, 5 characteristic, 1 not characteristic; while 12 did not coagulate in the recent tests, although all gave a positive coagulation when first tested; 7 of these characteristic, 5 not characteristic. When first studied, 19 were typical, 8 fairly typical.

23 cultures from normal cases were studied, 8 of these coagulated inulin not later than in first tests, 4 of these characteristic, 4 not characteristic; 2 coagulated later than in first tests, both characteristic; 13 did not coagulate at this time, although positive in original tests. Of the latter, 8 are characteristic, 5 not characteristic. When first studied, 13 were typical, 4 not characteristic and 6 atypical.

Of the 11 cultures from miscellaneous cases, 4 coagulated inulin not later than when first studied, 2 characteristic, 2 not characteristic; 2 coagulated later, 1 characteristic, 1 not, and 5 failed to coagulate, although positive in original tests, 4 of these characteristic, 1 not characteristic. In original tests, 6 were typical, 5 not characteristic.

No detailed account is given in these tables of original mannite coagulations. Only 8 of the 61 strains recently tested coagulated mannite,

5 from pneumonia cases, 2 from normal, 1 miscellaneous. Of 5 cultures from normal cases originally tested in mannite, 3 coagulated, 2 did not; all of these were negative in the recent tests.

Several of the strains now found to be negative for inulin, although positive when first tested, were inoculated into rabbits and mice in large doses. Of 9 mice inoculated, eight have died. From five of these, there are typical cultures, and capsules in heart smears, but so far, no coagulation of inulin. Of the rabbits, only one has died. From this rabbit, inoculated with N-91, there are typical pneumococcus cultures, capsules, and inulin coagulated on second day; mannite negative. Inulin and mannite cultures made from serum broth culture before inoculation into animal remain uncoagulated.

Cultures from 36, a typical pneumococcus, and from 47, a capsule coccus, both originally virulent, were carried on for many generations in serum broth, on rabbit's blood agar, horse blood agar, rat blood agar and mouse blood agar. In July and August, these were tested for virulence on rabbits, mice and rats. Both 36 and 47 were found to have decidedly lost virulence, 36 in greater proportion than 47, although in the original test 36 was one of the most virulent organisms isolated. In a further test of virulence made in October with cultures of this series, 36 was found to have entirely lost virulence for rabbits when inoculated in 4 c.c. doses, and was no longer virulent for mice unless given in large doses of a strong emulsion. 47 also now failed to kill rabbits in 4 c.c. doses; mice inoculated with 1/10 c.c. died, but the organism was not recovered at autopsy. Some difference in virulence was noticed in the cultures from these strains carried on upon different media, but on the whole, the results were irregular, and further tests would be necessary before drawing any definite conclusions. The coagulation of inulin and mannite has also been irregular in the cultures of these series. In earlier cultures all coagulated. Of those still living at the present time, the results of recent coagulation tests were as follows:

36—horse blood agar—Inulin coagulated in 24 hours; mannite not coagulated.

36—rabbit blood agar—Inulin coagulated in 24 hours; mannite not coagulated.

36—rat blood agar—Inulin coagulated in 48 hours; mannite not coagulated.

47—horse blood agar—Inulin coagulated in 24 hours; mannite coagulated in 8 days.

47—rabbit blood agar—Inulin not coagulated in several tests, though showing abundant growth, mannite also negative.

Organisms from the 47-rabbit blood agar series are decidedly atypical in morphology, and both in serum broth and plate cultures now show as much resemblance to streptococcus as to pneumococcus. One set of 36 cultures carried on among the regular laboratory stock strains now also shows a very atypical morphology, and fails to coagulate inulin. Further studies with cultures from these two cases are still in progress.

Virulence Tests—Made after Prolonged

36 1001

			Weight Grams.	Culture Inoculated.			Result.
				Amount.	Transfers since Isolation.	Transfer on Special Medium.	
1905.							
Serum broth.....	June 27	Rabbit..	910	.001 cc	73	18	Lived.
	June 27	Rabbit..	950	.00001 cc	73	18	"
	June 27	Mouse..001 cc	73	18	2 days.
	June 27	Mouse..0001 cc	73	18	8 "
Horse blood agar.....	June 27	Rabbit..	860	.001 cc	71	*17	6 "
	June 27	Rabbit..	910	.00001 cc	73	17	Lived.
	June 27	Mouse..001 cc	73	17	8 days.
	June 27	Mouse..00001 cc	73	17	6 "
Rabbit blood agar.....	June 27	Rabbit..	920	.001 cc	59	*15	8 "
	June 27	Rabbit..	960	.00001 cc	59	15	8 "
	June 27	Mouse..001 cc	59	15	8 "
	June 27	Mouse..00001 cc	59	15	6 "
	June 30	Mouse..001 cc	59	15	10 "
	June 30	Mouse..00001 cc	59	15	6 "
	June 30	Rat.....1 cc	■	15	Lived.
	June 27	Rat.....001 cc	59	15	6 days.
	June 27	Rat.....1 cc	59	15	13 "
	June 27	Rat001 cc	■	15	Lived.
Rat blood agar.....	June 27	Rabbit..	930	.001 cc	72	*17	"
	June 27	Rabbit..	850	.0001 cc	72	17	"
	June 27	Mouse..001 cc	72	17	8 days.
	June 27	Mouse..00001 cc	72	17	6 "
	June 27	Rat.....01 cc	■	17	13 "
Mouse blood agar.....	June 27	Rat.....001 cc	72	17	Lived.
	June 27	Rabbit..	900	.001 cc	73	17	"
	June 27	Rabbit..	800	.00001 cc	73	17	8 days.
	June 27	Mouse..001 cc	73	17	8 "
	June 27	Mouse..00001 cc	73	17	6 "

* Plus one serum broth culture inoculated.

Original virulence—

Mouse, .000001 cc—2 days.

Rabbit, .000005 cc—2 days.

Cultivation on Various Media.

471001

Series.	Date of Inoculation.	A	Inoc.	Culture Inoculated			Result.
					Transfer on Special Medium.		
	1905.						
Serum broth.....	June 30	Rabbit..	1040	4. cc	83	22	11 days.
	June 30	Rabbit..	1010	.1 cc	83	22	15 "
	June 29	Mouse..5 cc	81	20	1 day
	June 29	Mouse..1 cc	81	20	1 "
	June 29	Mouse..01 cc	81	20	1 "
Horse blood agar.....	June 26	Rabbit..	1080	4. cc	81	*21	Lived.
	June 26	Rabbit..	804	.1 cc	81	21	"
	June 26	Mouse..5 cc	81	21	2 days.
	June 26	Mouse..1 cc	81	21	2 "
	June 26	Mouse..01 cc	81	21	2 "
Rabbit blood agar.....	June 30	Rabbit..	1090	4. cc	89	*18	Lived.
	June 30	Rabbit..	1000	.1 cc	89	18	"
	June 30	Mouse..5 cc	78	*17	4 days.
	June 30	Mouse..1 cc	78	17	2 "
	June 30	Mouse..1 cc	78	17	4 "
	June 30	Rat.....01 cc	78	17	8 "
Rat blood agar.....	June 30	Rabbit..	1020	3. cc	77	*19	5 days.
	June 30	Rabbit..	1040	.1 cc	77	19	Lived.
	June 29	Mouse..5 cc	77	*17	1 day.
	June 29	Mouse..1 cc	77	17	6 days.
	June 29	Mouse..01 cc	77	17	4 "
	June 30	Rat.....	4. cc	79	*91	1 day.
	June 30	Rat.....1 cc	79	19	6 days.
	June 29	Rat.....	4. cc	77	*16	1 day.
	June 29	Rat.....1 cc	77	16	6 days.
Mouse blood agar.....	June 29	Rabbit..	1400	4. cc	80	*14	Lived.
	June 29	Rabbit..	1040	1. cc	80	14	"
	June 29	Mouse..5 cc	80	14	6 days.
	June 29	Mouse..1 cc	80	14	7 "
	June 29	Mouse..01 cc	80	14	14 "

* Plus one serum broth culture inoculated.

Original virulence—

Mouse, .00001 cc—2 days.

Rabbit, 4. cc—3 days.

*Summary of Inulin Tests Made January, 1906, upon Strains of Pneumococci
Isolated at Different Periods between November, 1904, and March, 1905,
Compared with Similar Tests Made Soon after Isolation. All
Positive in Original Tests.*

	Coagulated not later than in Original Test.		Coagulated later than in Original Test.		Not Coagulated this Time, though Coagulated in Original Test.		Total.
	Characteristic.	Not Characteristic.	Characteristic.	Not Characteristic.	Charac- teristic.	Not Char- acteristic.	
Pneumonia Cases.	8 ₁₀₀₁	18 ₁₁₂₂	15 ₁₁₁₂ 3 days later	22 ₁₀₀₁ 2 days later	4 ₁₀₀₁	20 ₁₁₂₂	
	*9 ₁₀₀₁	77 ₁₀₀₁	39 ₁₀₀₁ 2 "			21 ₁₁₂₂	
	13 ₁₀₀₁		*47 ₁₁₂₂ 2 "		62 ₁₁₁₂	36 ₁₀₀₁	
	66 ₁₀₀₁		57 ₁₁₂₂ 8 "		67 ₁₀₀₁	*47 ₁₀₀₁	
	56 ₁₁₁₂		83 ₁₀₀₁ 10 "		72 ₁₀₀₁		
	69 ₁₀₀₁				75 ₁₀₀₁	73 ₁₀₀₁	
	82 ₁₂₂₂				76 ₁₀₀₁		
				98 ₁₁₂₂			
	7	2	5	1	7	5	27
	9		6		12		
Normal Cases.	N. 41 ₁₁₂₂	N. 106 ₁₁₁₂	N. 89 ₁₁₂₂ 8 days later		N. 23 ₁₁₂₂	*N. 10 ₁₁₁₂	
	N. 124 ₁₀₀₁	N. 111 ₁₀₀₁			N. 51 ₁₁₂₂	N. 11 ₁₁₂₂	
	N. 135 ₁₁₂₂	N. 127 ₁₁₁₂	N. 99 ₁₁₂₂ 9 days later		N. 52 ₁₁₂₂	N. 43 ₁₁₂₂	
	N. 136 ₁₂₁₂	N. 113 ₁₀₀₁			N. 59 ₁₁₂₂	N. 45 ₁₀₀₁	
						N. 107 ₁₁₁₁	
					N. 91 ₁₂₂₂		
					N. 100 ₁₁₂₂		
				N. 109 ₁₁₁₂			
				N. 130 ₁₁₁₂			
	4	4	2	0	8	5	23
	8		2		13		
Miscellaneous Cases.	C. & D. 4 ₁₀₀₁	C. 23 ₁₁₁₂ Me. 1 ₁₁₂₂	C. 5 ₁₁₂₂ 1 day later	{ Me. 2 ₁₁₁₂ 3 days later }	C. & D. 8 ₁₀₀₁	*C. 4 ₁₁₂₂	
	Q. 5 ₁₁₂₂				T. 3 ₁₁₂₂		
						Q. 3 ₁₀₀₁	
					C. 23 ₁₁₂₂		
	2	2	1	1	4	1	11
	4		2		5		
Total.....							61

* Pneu ococcus Mucosus.

PNEUMOCOCCUS STRAINS.

Morphology and Inulin Coagulations January, 1906, Compared with Similar Tests Made Soon After Isolation (from November, 1904, to August, 1905).

Pneumonia Cases.

	* Original Tests.				Tests made in January, 1906.			
	Morphology.	Capsule.	Virulence.		Inulin Coag.	Inulin Coag.	Mannite Coag.	Plate.
			Mouse.	Rabbit.				
4/1001	Large Typ. Pn. . .	—	1/2 c.c., alive.	3 c.c., alive.	+ 3 days	— 14 days	— 14 days	Fairly Typ. Pn.
8/1001	Typ. Pn.	—	Not inoc.	Not inoc.	+ 2 "	+ 2 "	— 14 "	Typ. Pn.
9/1001	Typ. Caps. Coccus	+	1/2 c.c., alive.	3 c.c., alive.	+ 2 "	+ 2 "	+ 5 "
13/1001	Typ. Pn.	—	1/200 c.c., 1 day.	1/10 c.c., 2 days.	+ 2 "	+ 2 "	+ 2 "
15/1112	"	+	1/1,000 c. c., 2 days.	3 c.c., alive.	+ 1 day.	+ 4 "	— 14 "	Fairly Typ. Pn.
18/1122	"	?	Not inoc.	1/10 c.c., 7 days	+ 2 days	+ 2 "	— 14 "	Not characteristic....
20/1222	Fairly Typ. Pn. . .	+	+ 1 day.	— 14 "	— 14 "	"
21/1122	"	—	Not inoc.	4 c.c., 1 day.	+ 1 "	— 14 "	— 14 "	Atypical.
21/1122	"	—	"	4 c.c., 1 day.	+ 2 days	+ 4 "	— 14 "	Not characteristic....
22/1001	Typ. Pn.	—	1/1,000,000 c.c., 2 days	1/5,000, 2 days.	+ 1 day.	— 14 "	— 14 "	Very atypical.
36/1001	Fairly Typ. Pn. . .	—	Not inoc.	1/10, 4 days.	+ 4 days	+ 2 "	+ 5 "	Fairly typical.
39/1001	Typ. Caps. Coccus	+	1/100, 2 days.	4 c.c., alive.	+ 3 "	— 14 "	— 14 "	Atypical.
47/1001	"	+	1/100,000 c.c., 2 days	4 c.c., 3 days.	+ 1 day.	+ 3 "	+ 3 "	Typ. Caps. Coccus...
47/1122	"	?	1/100, 25 days.	3 c.c., alive.	+ 2 days	+ 2 "	— 14 "	Typ. Pn.
56/1112	Typ. Pn.	Indicated.	1/100, 1 day.	4 c.c., 1 day.	+ 2 "	+ 10 "	— 14 "	"
57/1122	"	—	1/2 c.c., 3 days.	3 c.c., alive.	+ 4 "	— 14 "	— 14 "	Not characteristic....
62/1112	Fairly Typ. Pn. . .	—						Typical Pn

* Original Tests.				Tests made in January, 1906.			
Morphology.	Capsule.	Virulence.		Inulin Coag.	Mannite Coag.	Plate.	Smears from Serum Media.
		Mouse.	Rabbit.				
66/1001	—	Not inoc.	Not inoc.	+ 4 days	— 14 days	Atypical.	Atypical.
67/1001	—	"	"	+ 2 "	— 14 "	Typ. Pn.	Not characteristic.
69/1001	—	"	"	+ 2 "	— 14 "	Fairly typical.	"
72/1001	—	"	"	+ 2 "	— 14 "	"	Fairly typical.
73/1001	—	"	"	+ 3 "	— 14 "	Atypical.	Atypical.
75/1001	—	"	"	+ 2 "	— 14 "	Typ. Pn.	
76/1001	—	"	"	+ 1 day.	— 14 "	Fairly typical Pn.	Not characteristic.
77/1001	—	"	"	+ 2 days	— 14 "	Not characteristic.	
82/1322	+	"	1/10 c.c., 2 days	+ 3 "	— 14 "	Fairly typical.	Not characteristic.
83/1001	—	"	Not inoc.	+ 1 day.	— 14 "	"	Fairly typical.
98/1122	+	"	4 c.c., alive.	+ 1 "	— 14 "	"	Not characteristic.

* Special stains for capsule not made until after passage through animals. Where no capsule was found with 1001, the entries refer to staining with ordinary stain.

Miscellaneous Cases.

Morphology.		• Original Tests.				Tests made in January, 1906.			
		Capsule.	Virulence.		Inulin Coag.	Inulin Coag.	Mannite Coag.	Plate.	Smears from Serum Media.
			Mouse.	Rabbit.					
C. & D. 4/1001	Typ. Pn.....	Indic.	+ 2 days	+ 2 days	- 14 days	Fairly typical.....	Very typical.
C. & D. 8/1001	"	"	+ 2 "	- 14 "	- 14 "	Typical Pn.....	Fairly "
T. 3/1122	"	-	Not inoc.	3 c.c., 1 day	+ 1 day	- 14 "	+ 10 "	"	Very "
Q. 3/1001	"	-	"	Not inoc.	+ 1 "	- 14 "	- 14 "	"	Not characteristic.
Q. 5/1122	Typ. Pn	-	"	1/10 c.c., 5 days	+ 1 "	+ 1 day	- 14 "	Maj. typ. Pn.....	Typical Pn.
C. 4/1122	Typ. Caps. Coccus	+	1/10000, 4 days	1/10 c.c., 8 "	+ 9 days	- 14 days	- 14 "	Atypical.....	Fairly typical.
C. 5/1122	Not char. Pn.....	-	Not inoc.	3 c.c. alive.	+ 2 "	+ 3 "	- 14 "	Typical Pn.....	"
C. 23/1112	"	+	"	4 c. c., 8 days..	+ 1 day	+ 1 day	- 14 "	Atypical.
C. 23/1122	"	-	"	4 c.c., 8 "	+ 1 "	- 14 days	- 14 "	Typical Pn.....	Fairly typical.
Me. 1/1122	"	+	1/1000, 3 days	1/1000, 3 "	+ 7 days	+ 7 "	- 14 "	Not characteristic	Not characteristic.
Me. 2/1112	"	+	1/1000, 2 "	3 c.c., 1 day	+ 1 day	+ 4 "	- 14 "	"	"

* Special stains for capsule not made until after passage through animals. Where no capsule was found with 1001, the entries refer to staining with ordinary stains.

Normal Cases.

		* Original Tests.				Tests made in January, 1906.			
	Morphology.	Capsule.	Virulence.		Inulin Coag.	Inulin Coag.	Mannite Coag.	Plate.	Smears from Serum Media.
			Mouse.	Rabbit.					
N. 10/1112	Typ. Caps. Coccus	+	1/1000, 2 days.	Not inoc.	+ 2 days	-14 days	-14 days	Not characteristic....	Not characteristic.
N. 11/1122	Large Typ Pn....	+	1/100, 4 days.	"	+ 2 "	-14 "	-14 "	"	"
N. 23/1122	Typ. Pn.....	+	Not inoc.	4 c. c.	+ 3 "	-14 "	-14 "	Typical Pn.....	"
N. 41/1122	Pn. Caps. Coccus.	+	"	4 c.c. 1 d., alive	+ 5 "	+ 3 "	-14 "	"	Fairly typical.
N. 43/1122	Atyp. Pn.....	-	"	4 c. c., 2 days.	+ 4 "	-14 "	-14 "	Not characteristic....	Not characteristic.
N. 45/1001	Not char. Pn.....	-	"	4 c. c., 12 "	+ 4 "	-14 "	-14 "	Atypical.....	"
N. 51/1122	Atyp. Pn.....	Indicat.	"	4 c. c., 3 "	+ 5 "	-14 "	-14 "	Typical Pn.....	Fairly typical.
N. 52/1122	"	-	"	4 c. c., 11 "	+ 5 "	-14 "	-14 "	Fairly typical.....	"
N. 59/1122	Typ. Pn.....	Indicat.	"	1/10 c. c., 6 days	+ 8 "	-14 "	-14 "	"	"
N. 88/1122	"	?	"	4 c. c., 3 days.	+ 5 "	-14 "	-14 "	Fairly typical.....	Typical Pn.
N. 89/1122	"	-	"	4 c. c., 2 "	+ 1 day	+ 9 "	-14 "	Very typical.....	"
N. 91/1222	"	+	"	1/1000 c. c., 3 d.	+ 1 "	-14 "	-14 "	Fairly typical.....	Fairly typical.
N. 99/1122	"	+	"	4 c. c., 1 day.	+ 1 "	+ 10 "	-14 "	Typical Pn.....	"
N. 100/1122	Not char. Pn.....	+	"	1/10 c. c., 1 day	+ 3 days	-14 "	+ 11 "	Typical Pn.
N. 106/1112	Typ. Pn.....	+	1/100000, 2 days	3 c. c., alive.	+ 1 day	+ 1 day	-14 "	Not characteristic....	Not characteristic.
N. 107/1111	Not char. Pn.....	+	Not inoc.	Not inoc.	+ 3 days	-14 days	-14 "	"	Fairly characteristic.
N. 109/1112	Atyp. Pn	-	1/100, alive.	4 c. c., alive.	+ 3 "	-14 "	-14 "	Fairly typical.....	Not characteristic.

N. 111/1001	Typ. Pn.....	—	Not inoc.	Not inoc.	+	+ 3 days	—10 days	Not characteristic....	Not characteristic.
N. 113/1001	Atyp. Pn.....	—	"	"	+ 1 day.	+ 1 day.	—14 "	"	"
N. 124/1001	"	—	"	"	+ 1 "	+ 1 "	—14 "	Fairly typical.....	Fairly typical.
N. 130/1112	Typ. Pn.....	?	1/100 c.c., alive	3 c. c., alive.	+ 3 days	—14 days	+ 1 day.	"	"
N. 135/1122	"	+	1/100000, 2 days	4 c. c., 6 days.	+ 1 day.	+ 1 day.	—14 days	Not characteristic....	"
N. 136/1212	"	+	1/100 c. c., 2 d.	3 c. c., 5 "	+ 14 days	+ 1 "	—14 "	Typical Pn.....	Typical Pn.

* Special stains for capsule not made until after passage through animals. Where no capsule was found with 100x, the entries refer to staining with ordinary stains.

THE APPLICATION OF THE REACTION OF AGGLUTINATION TO THE PNEUMOCOCCUS.

BY KATHERINE R. COLLINS, M. D.,

Bacteriologist.

The following report is a part of the work on pneumonia as planned by the Commission for the Investigation of Acute Respiratory Diseases, and by the Department of Health.

Normal serum of various animals differs greatly in its tendency to agglutinate many strains of pneumococci. Thus rabbit serum generally gave negative results, while sheep and horse serum reacted slightly in a few instances, and the serum of one goat of four tested agglutinated a number of strains of pneumococci in dilutions of 1.10.

Neufeld, Clairmont, Landsteiner, Wadsworth, Heyrovsky, and others have succeeded in producing agglutinins for pneumococci in the animal body through immunization.

Gorgano and Fattori state that agglutination of *Diplococcus pneumoniae* with the blood of patients suffering from infection with this organism is constant, that it persists for some time after recovery of the patient, and that the reaction is more marked if the homologous organism be used. The highest reaction obtained by them was, however, in a 1.10 dilution.

We have found that the sera of normal individuals in many instances reacts with the various strains of pneumococci in dilutions of 1.2 and 1.10, hence a higher reaction would be required than was found by these observers for diagnosis.

Glucose broth has been generally recommended as the medium best adapted for making agglutination tests with the pneumococcus, but as the organism quickly dies out in the presence of the excess of acid produced by the fermentation of the sugar, it can be carried through one generation only on this medium, unless it is transferred before the acidity has increased sufficiently to destroy the growth. This fact makes the broth unsuitable for the work.

Marshall and Knox¹ and Morello have shown that the typhoid bacillus loses its agglutinability when grown for some time in an active immune serum. Dr. Park and I have demonstrated the same to be true for the bacillus of dysentery when grown in its immune serum, and we were also able to show, further, that the agglutinability could be restored by long cultivation upon suitable media.

As the presence of serum constituents in the medium is required for the continuous growth of the pneumococcus, it may be assumed from the above facts that the agglutinability of the organism might at least be lowered by long cultivation upon a medium containing even very small amounts of these inhibitory substances. This assumption was borne out by several tests made with an organism taken on the one hand directly from a fresh rabbit-blood-agar culture, and on the other from a culture in calcium broth² one generation old and several generations old. The last culture gave the best reaction, while the culture from the blood-agar gave the poorest reaction.

To eliminate this source of error, diluted sheep or hog serum, as suggested by Dr. Park, was boiled to destroy any inhibitory substances present, and added to broth or agar as the case required. Cultures obtained from media containing these sera, when transferred to calcium broth, usually gave a homogeneous growth with much less tendency to spontaneous agglutination than is seen with cultures grown in calcium-glucose broth. Heating the organism to 70 degrees C. for 15 minutes does not affect its agglutinability. Heating the serum to 85 degrees for 15 minutes, however, destroys the agglutinins both for the pneumococcus and the pneumococcus mucosus.³

Several methods of immunization were tried. The one which gave the best results is represented by the following example:

Feb. 15—A rabbit was given subcutaneously 3 c.c. of a culture grown for 48 hours in broth, to which a few drops of defibrinated blood were added, heated previously to 60° C. for 30 minutes.

Feb. 28— 5 c.c. of a similar culture administered.

¹ The studies of Marshall and Knox, so far as I know, have not as yet been published.

² The fact that the addition of calcium carbonate to culture media neutralizes the acid formed by the fermentation of sugar during bacterial growth has been recognized for some time. The application of this reaction to the growth of the pneumococcus was suggested independently by Bolduan and Hiss. The former recommended the addition of bits of marble to plain broth, the latter used calcium carbonate in the form of powder in glucose broth. (See Dr. Bolduan's paper, page 137).

³ A description of this organism will be found in the article by Park and Williams (page 91).

- March 8— $\frac{1}{2}$ c.c. of a living culture of the same organism was given.
 " 13— 1 c.c. of a living culture of the same organism was given.
 " 24— 5 c.c. of a calcium-broth culture, heated 70° C. for 15 minutes, given.
 April 1— 5 c.c. of a calcium-broth culture, heated 70° C. for 15 minutes, given.
 " 8— 5 c.c. of a calcium-broth culture, heated 70° C. for 15 minutes, given.
 " 15—10 c.c. of a calcium-broth culture, heated 70° C. for 15 minutes, given.
 " 25—10 c.c. of a calcium broth culture, heated 70° C. for 15 minutes, given.
 May 1—10 c.c. of a calcium-broth culture, heated 70° C. for 15 minutes, given.
 " 8—15 c.c. of a calcium-broth culture, heated 70° C. for 15 minutes, given.
 " 15—15 c.c. of a calcium-broth culture, heated 70° C. for 15 minutes, given.
 " 21—Animal bled and the serum tested with the homologous organism which it agglutinated in a dilution of 1:200.
 May 22—An emulsion heated to 70° C. for 15 minutes from four heated serum-agar plates was injected subcutaneously. Animal dead on the following day.

Hanging drops were chiefly relied on for ascertaining the reactions, though in many instances these were controlled by the macroscopic method and the contents of the tubes examined microscopically after reaction had taken place.

The sources of error seem about equal in the two methods, while the hanging drop has the advantage of shorter time limit of reaction, and of easy recognition of contamination.

With the pneumococcus the tube method generally indicates a higher microscopical reaction than the hanging drop. This is contrary to tests made with the dysentery and typhoid bacilli, and is explained by the fact that in the former case the free organisms must be present in great numbers to cloud the supernatant fluid, whereas in the latter a comparatively small number of free bacilli may render the fluid turbid, so that in the case of the pneumococcus a good reaction viewed macroscopically may become only a fair reaction when viewed microscopically.

Neufeld states that the various strains of the pneumococcus agglutinate alike, an observation probably due to the low reactions obtained by him, since his maximum reaction was 1:50. My work has shown great irregularity in this respect, the serum of an animal immunized with one strain of pneumococcus agglutinating only seven organisms out of seventy tested in dilutions equalling the reaction (1:200) with the homologous organism. Four strains reacted in dilutions of 1:10, eleven in 1:2, while the remaining organisms were entirely negative.

The serum of a second immunized animal agglutinated the homologous organism in dilution of 1:100, while other strains were affected in less dilutions or not at all.

Since the first publication of this article A. Kindborg from Fraenkel's Laboratory at Halle has confirmed these results.

TABLE I.

Agglutination Tests with the Serum of a Sheep¹ Injected for a Period of Three Months with Pneumococcus No. 36.

Dilution.	2	10	20	50	100	Control.
Typical pneumococcus 36..	++	++	++	++	+ I	—
Typical pneumococcus 14..	—	—	—
Typical pneumococcus 4..	++	I	I
Typical pneumococcus 16..	++	—	—
Typical pneumococcus 18..	—	—
Typical pneumococcus 33..	—	I
Atypical pneumococcus 2..	++	—	—
Atypical pneumococcus 66..	+I	—	I
Pneumococcus mucosus 47.	—	—

¹ For the details of the immunization of the sheep, the paper of Drs. Park and Williams is to be consulted.

The remaining sixty-one organisms tested reacted some in dilution of 1:2 and others not at all, thus emphasizing the distinction of pneumococcus No. 36 from the other strains in regard to its power to produce agglutinins.

There is apparently a difference in the agglutinability of the pneumococci, some strains uniformly reacting much more readily than others in normal and immune sera.

Two strains of pneumococci which showed good agglutination with several active sera failed to produce agglutinins to any extent in the animal body for themselves or other strains of pneumococci. Three rabbits and a goat were inoculated without results with one strain, and two rabbits and a horse with the other; and as the same kind of animals was immunized under the same conditions with other strains with good results, this irregularity would seem to indicate a peculiarity of the organism rather than of the animals injected.

Another observation of interest, but one which has not been carried far enough on account of insufficient time to establish definite conclusions, is certain reactions obtained with a streptococcus serum and absorption experiments made with this organism.

A young goat which was immunized with a strain of streptococcus yielded a serum which agglutinated a few pneumococci and its own culture in dilutions of 1:10.

Pneumococcus 66, which coagulated inulin-serum water late, when first isolated and several months later not at all absorbs the agglutinins for several pneumococci from a typical pneumococcus immune serum. This culture is the only one of the pneumococci that has its agglutinins taken out of the above pneumococcus immune serum by the streptococcus; it produces agglutinins in the animal body for itself and many of the pneumococci. These reactions suggest the possibility of the occurrence of intermediate types of organisms between pneumococci and streptococci.

EXHAUSTION EXPERIMENTS.

The extreme sensitiveness of the pneumococcus to changes of conditions not readily determined brings about variation in the behavior

of this organism which proved a serious factor in the application of the agglutination reaction and in the interpretation of the results obtained. To eliminate as far as possible any errors arising from this instability, the exhaustion experiments were conducted in groups, each group covering as many observations as practical, in order to insure uniform conditions for a number of tests.

In the exhaustion experiments a slight loss of agglutinins has generally been observed. This loss occurs whether the organism used for absorption is an homologous or a related one or of a foreign type. This fact points to the cause of the loss lying outside of the presence of the organism. The loss is readily estimated on account of its uniformity, and in no way affects the determination of the amount of absorption excepting where a strain reacts only in low dilutions. In this case the disappearance of the agglutinins cannot be ascribed with certainty to the organism used for absorption, and the establishment of relationship by the absorption method is not possible in these instances.

Testing the reaction of the meningitis coccus in antipneumococcus serum, Sorgente failed to obtain agglutination with a number of strains. We failed to absorb the agglutinins from a serum agglutinating several strains of pneumococci in dilution of 1:200 with a culture of the diplococcus of meningitis.

As shown in Table II., the power of the serum to agglutinate pneumococcus Nos. 14 and 72 in equally high dilutions with the homologous organism, and by absorption that the agglutinins are group agglutinins for Nos. 14 and 72 and both group and specific agglutinins in the case of No. 47, the homologous organism is explained very readily by the fact that the two types of agglutinins constantly vary in ratio both in different animals and at different periods of inoculation; the group agglutinins even exceeding at times the specific ones.

The increase of agglutinins for different strains of the pneumococcus mucosus in the serum of an animal inoculated with one of the typical pneumococcus strains, and the results obtained by the absorption of these agglutinins, separate them into a distinct variety from the majority of other pneumococci.

The similar results obtained, as indicated in the following table, by absorption with pneumococcus mucosus No. 47 and pneumococcus No. 4 (the latter organism when studied not producing the characteristic capsule and gelatinous colonies), suggest that these cultural attributes may be lost while the agglutinative affinity is still retained.

TABLE

Serum of a Sheep Injected with *Pneumococcus Mucosus* (Williams), Showing the *Pneumococci*, a Strain of *Streptococcus*.

		Before Exhaustion							
			6	10	20	50	100	200	Control
Pneumococcus mucosus	{	25	++	++	++	++	++	+ I	- +
		47	++	++	++	++	++	++	-
		C1	++	++	++	++	+ I	-	-
		C4	++	++	++	++	++	++	- +
Atypical pneumococci.	{	2	++	++	++	+	-
		72	++	++	++	++	++	I	I
Typical pneumococci..	{	4	++	++	++	++	+ I	+	- +
		14	++	++	++	++	++	+	-
		11	- +	-
		36	-	-
Typical pneumococci	{	56	I	I
		Simple cold 2 C. & D.	I	I
		Normal.... N. 46	+ I	+	- +	I

Part of the remaining organisms tested reacted in dilutions of 1:2 and part failed to react even in + + complete agglutination ; + 1 not quite complete ; + good ; 1 trace ; - + fair ; - negative.

II.

Agglutination Index Before and After Absorption with Its Own Group, Typical and Bacillus Typhosus Respectively.

After Exhaustion with Pneumococcus No. 47.		After Exhaustion with Pneumococcus No. 14.				After Exhaustion with Pneumococcus No. 4.		After Exhaustion with Streptococcus longus.					After Exhaustion with B. typhosus.			
6	20	6	20	50	100	6	20	6	20	50	100	200	6	20	50	100
- +	- +	++	++	++	- +	- +	- +	++	++	++	++	- +	++	++	++	+
-	-	++	++	++	+	I	I	++	++	++	++	+	++	++	++	++
+	-	++	++	++	- +	- +	- +	++	++	++	+	- +	++	++	++	+
- +	- +	++	++	++	+	-	-	++	++	++	+	- +	++	++	++	- +
++	+	++	++	+	I	-	-	++	++	- +	I	..	++	++	++	..
+	-	+	- +	I	..	- +	- +	++	++	++	I	..	++	++	+	..
++	- +	- +	- +	- +	..	-	-	++	++	++	+	..	++	++	++	2
I	I	- +	I	I	I	++	++	++	+	..	++	++	++	I

this low dilution.

TABLE

The remaining organisms tested reacted as in Table II. The above tests were made simultaneous. The irregularity of the pneumococci in their behavior to the agglutination reaction is amply justified based upon the morphological and culture characteristics of the organisms. Atypical pneumococci in their agglutination reactions.

The different degrees of partial exhaustion of agglutinins for normal culture No. 46 suggests as to the streptococcus longus.

III.

Serum of a Horse Injected during a Period of Three Months with Pneumococcus No. 14 before and after Exhaustion with Pneumococci Nos. 22 and 72.												
Before Exhaustion.					After Exhaustion with Pneumococcus No. 22.				After Exhaustion with Pneumococcus No. 72.			
6	20	50	100	200	6	20	50	100	6	20	50	
+	I	+	—	—	
++	++	++	+	++	++	+	I	
++	++	+	+	I	I	—+	—+	
++	++	++	++	+I	+I	+	+I	+I	
++	++	—+	—+	++	++	—+	—+	—+	
++	—+	++	—	+I	—+	
++	++	++	++	++	++	I	I	

eously, the same cultures being used throughout.
indicated by the tables. The interaction of typical and atypical cultures does not bear out the classi-
pneumococci 2, 66 and 72 differ constantly from each other, and yet correspond with several of the
the existence of agglutinins common to this culture and several other strains of pneumococci, as well

The ability of the pneumococcus mucosus group to produce common agglutinins for some pneumococci, and the fact that the streptococcus failed to affect through absorption their agglutinins, would indicate a closer relation of this variety to the pneumocci than to the streptococci.

CONCLUSIONS.

I.—Pneumococci by reason of their agglutinating properties exhibit a tendency to separate into numerous groups similar to streptococci.

II.—The pneumococcus mucosus forms a distinct and consistent variety. The production by it of common agglutinins for some strains of pneumococci would seem to indicate a relationship between the two classes of organisms.

III.—The agglutinating substances in the serum of immunized animals were demonstrated by absorption tests to consist of specific and group agglutinins in cases where the agglutinins were sufficiently developed to make use of this method.

IV.—The pneumococci seem to show marked differences in their ability to undergo agglutination.

V.—There was considerable uniformity of reaction of the various strains in low dilutions, but this uniformity is not continued as the animal becomes more highly immunized.

VI.—At present a definite relation between the agglutination reaction and other characteristics of the pneumococci is not established excepting in the case of the pneumococcus mucosus.

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**THE ADDITION OF CALCIUM SALTS TO NUTRIENT
BROTH. A RELIABLE AND CONVENIENT METHOD
FOR GROWING THE PNEUMOCOCCUS, MEN-
INGOCOCCUS, AND CERTAIN OTHER
BACTERIA.***

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During the course of some work on the pneumococcus carried on at the research laboratory the need was felt for a culture medium which did not contain any animal body fluids (ascitic fluid, blood serum, etc.). Plain broth was found almost worthless for the pneumococcus, for while one or two generations might grow if planted for blood agar, the growth soon died out, making continued transplantations from broth to broth impossible. Nor did careful neutralization of the broth to phenolphthalein nor even slight alkalization yield any better results. The addition of glucose, as is well known, does cause a luxuriant growth of the pneumococcus, but the production of acid in this case is so great that the cultures die very quickly and therefore need transplantation every few days. The writer felt that it might be possible to neutralize most of this acid as it was produced and thus obtain a broth possessing all of the advantages of glucose broth without its disadvantages. For this purpose calcium carbonate was employed, at first in the form of powder and later, because of several advantages, in the form of pieces of marble.

A number of strains of pneumococcus were subjected to a critical comparative test in a series of about twenty different culture media. On transplanting the cultures every day or two it was soon found that certain of the media, although they yielded a profuse growth, were unreliable; i. e., a growth was not always obtained with each inoculation, even though the culture was still alive. In order to carry such a culture on it was often necessary to reinoculate from the same culture, using, however, a larger amount. Among these unreliable media were glucose broth and glucose broth plus calcium carbonate. Curiously enough *plain broth with marble, but without glucose*, yielded a moderate growth unfailingly with each transplantation.

* Read before the New York Pathological Society, April 12, 1905.

On testing the acidity of the cultures in the various media it was found that large quantities of acid were developed in media containing glucose alone, or glucose and calcium carbonate, while small quantities of acid developed in ascitic broth or even in plain diluted ascitic fluid. The cultures in plain broth plus marble on the other hand were usually neutral, though a very small development of acid was now and then observed.

When plain broth is inoculated from an ascitic broth culture of pneumococcus, there frequently is not only no increase in organisms, but an actual decrease. When marble is present there is a growth right from the start. This fact cannot be reconciled with the assumption that the marble acts only by neutralizing acids formed. On reviewing these data Dr. Park suggested that the virtue of the marble might reside in the calcium and not merely in its neutralizing power. Two other salts of calcium, the chloride and the sulphate, were therefore tested. The former was used in solution, one part to two thousand of broth, the latter was used in pieces like the marble already mentioned. My experiments with these two salts indicate that *it is the calcium element, in part at least, which favors the growth of the pneumococcus.* Calcium carbonate (marble) and calcium sulphate (gypsum) are ordinarily regarded as insoluble in water. Nevertheless, a small trace of calcium must pass into solution in order to account for this effect on the growth of the pneumococcus. Since marble also serves to neutralize some of the acid formed, it would seem preferable to the other salts of calcium. Further experiments, however, are necessary to determine this point.

As already stated pneumococcus cultures in media containing glucose die off very quickly. It was not found that the viability of such cultures was increased by the presence of the marble. This is not surprising, for our titrations showed that the marble failed to neutralize considerable quantities of acid. On the other hand, in plain broth plus calcium carbonate (marble broth) the cultures lived just about as long as those in ascitic broth. Out of three different strains tested, one was still alive at the end of thirty days. Out of four, three were still alive at the end of seventeen days.

A point of considerable importance is the effect produced by continued growth in a medium on the virulence of the organism. The cultures tested for this purpose had been grown for fourteen consecutive generations in marble broth. None of them seemed to have lost any virulence when compared with an ascitic broth culture made directly from the blood agar stock cultures.

A peculiarity of the marble broth cultures observed several times was the formation of short chains, while a control culture in ascitic broth showed only two's. Besides this, the absence of anything indicative of a capsule makes this medium a poor one for bringing out the characteristic morphology of the pneumococcus. On the other hand, cultures of the so called *streptococcus mucosus capsulatus* when grown in this medium take on a form practically the same as that of an ordinary pneumococcus.

In preparing this medium, marble is broken up into small pieces in an iron mortar. The finer pieces are removed by screening through an ordinary wire crate. The others, about the size of small dice, are washed in water and one or two pieces placed into each tube. The tubes are then filled in the usual way with plain broth and sterilized in the autoclave.

From the foregoing it will be seen that marble broth is an extremely reliable medium for the pneumococcus, and can in very many cases replace ascitic broth. Its use obviates not only the tedious collection of ascitic fluid, but also the careful fractional pasteurization which ascitic broth requires.

Marble broth can be used also for cultures of the meningococcus. The medium does not produce quite so luxuriant a growth as ascitic broth, but seems to be very reliable.

THE COMMUNICABILITY OF CEREBRO-SPINAL MENINGITIS AND THE PROBABLE SOURCE OF CONTAGION.

PART OF AN INVESTIGATION OF CEREBRO-SPINAL MENINGITIS CARRIED OUT UNDER THE AUSPICES OF THE SPECIAL COMMISSION OF THE DEPARTMENT OF HEALTH OF NEW YORK CITY.*

BY CHARLES BOLDUAN, M. D.,

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Epidemic cerebro-spinal meningitis, so far as history is concerned, seems not to have been clearly observed prior to 1800. In the histories of the great epidemics of Europe, from the thirteenth century on, symptoms are described which almost certainly point to this disease. An interesting account of these early epidemics is given by Webber¹. Since 1800 the disease has appeared in four large epidemic periods. Hirsch² divides these periods as follows: the first, from 1805 to 1830, shows the disease more general in the United States, though there are also isolated epidemics in various places in Europe. In the second period, from 1837 to 1850, meningitis became prevalent in widespread epidemics in France, Italy, Algeria, the United States and Denmark. During the third period, from 1854 to 1875, the malady reached its widest diffusion throughout most of Europe, the adjoining countries of nearer Asia, the United States and some parts of Africa and South America. The fourth period, from 1876 to the present time, is a return to merely casual epidemic outbreaks or to more or less considerable groups of cases here and there within its former limits. It is to be noted, however, that during this period there is not a year wholly free from epidemics in some part of the world.

These four periods have been critically examined by Jaeger,³ who calls attention to the fact that the interval between the periods is growing shorter thus—seven, four and one year respectively—and that as our knowledge of the disease is becoming more definite and the cases are more carefully reported, one finds that epidemics of this disease have never entirely died out. Since the spontaneous origin of living disease

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germs is out of the question, Jaeger believes that such long intervals as that of seven years between the first and second period can only be explained "either by assuming that the virus has extraordinary powers of life outside the body, or that the virus is kept alive by being transmitted from one individual to another *without giving rise to epidemics*." The former possibility may be dismissed, for practically all authorities agree that the organism certainly does not possess very great powers of resistance. In fact, most authorities have found it to possess but little vitality. We must, therefore, look upon the sporadic cases as some of the connecting links between the epidemics and must also seek for the organism in individuals who are not infected.

Geographical and Seasonal Distribution—A careful study of the geographical distribution shows that the disease occurs mostly in the north temperate zone, although it has been observed as high north as Iceland and as far south as Java. Of 182 European epidemics, 24 were in October and November, 46 in May, 24 in June and July, 10 in August and September. In Sweden, of 417 local outbreaks, 311 were in winter and 106 in summer.⁴ Of 85 epidemics in the United States, 37 occurred in winter, 18 in winter and spring, and 23 in spring.² The disease has occurred in perfectly mild winters, such as those at Metz in 1839-40, in Italy in 1839-40 and 1840-41 in Indiana in 1862-63 and in Kentucky in 1866. Some epidemics in fact have not shown themselves until summer, as at Bordeaux in 1839, Toulouse in 1842, Dublin in 1850 and Cracow in 1874. When meningitis was epidemic in Asia Minor in 1868-70 it came to an end in Magnesia just when severe cold set in; but it showed itself at Smyrna in the spring under very high temperature.

Etiology—It is now generally conceded that the etiological factor in most of the cases of epidemic cerebro-spinal meningitis is the diplococcus described by Weichselbaum⁵ in 1887, and now commonly called the meningococcus Marchal⁶ analyzed 513 cases of cerebro-spinal meningitis. He divides the cases into primary sporadic and epidemic cases. In the former there were 95 cases, of which 48 showed the meningococcus, 40 the pneumococcus, and 7 various other organisms. Among the second class of cases, 418 in number, 307 (73 per cent.) were associated with the meningococcus, 67 (16 per cent.) with the pneumococcus, and 44 (10 per cent.) with various other bacteria.

Schottmuller⁷ examined 49 cases, finding the meningococcus of Weichselbaum 43 times. In three of the cases there was mixed infection, once with tubercle bacillus, once with pneumococcus and once with *streptococcus mucosus*.

In the literature of meningitis, especially shortly after the discovery of the organism and before bacteriological diagnosis had been very much developed, a number of instances are recorded in which epidemics of cerebro-spinal meningitis were observed in which the infecting micro-organism was other than the meningococcus. Some of these appear to have been epidemics of pneumococcus meningitis (Panienski,⁸ Quadu,⁹ Weichselbaum, cited in Schottmüller⁷). In others the statements of the authors are so conflicting that one hesitates to accept them (Bonome¹⁰). It must always be remembered that cases of pneumococcus and of streptococcus meningitis are fairly common, and that they will, naturally, occur also during an epidemic of meningococcus meningitis.

As said above, however, there is no doubt that the large majority of cases of epidemic cerebro-spinal meningitis are associated with the meningococcus.

In an excellent study of 119 cases of cerebro-spinal meningitis which occurred in Boston Councilman¹¹ says that "in a question of the probability of transmission of an infectious disease and the ways in which the organism causing it can pass from the lesions of the disease to the outside; further, the viability of the organisms and the possibility of their leading a saprophytic existence."

Occurrence of the Meningococcus—It is unnecessary here to go into the occurrence of the meningococcus in the meningeal exudate and in the fluid obtained by lumbar puncture. So far as the epidemiology of the disease is concerned, this localization of the organism in the interior of the skull and spinal canal is without special interest.

Councilman found Gram-negative diplococci in the nasal secretion of a number of patients suffering from meningitis. These organisms were not, however, isolated in pure culture and further identified. The same author isolated one culture from the tonsils of a patient with cerebro-spinal meningitis, and this proved to be a true meningococcus. Schiff¹² examined the nasal secretion of 27 healthy persons, finding an

intracellular diplococcus in 7. Of these 7, 3 were identified as meningococci by Weichselbaum. Kiefer,¹³ while working with a culture of meningococcus, was seized with a coryza. He thereupon succeeded in isolating a meningococcus from his nasal secretion. Lord¹⁴ also demonstrated the organism in the nasal secretion of a patient which he examined. Albrecht and Ghon¹⁵ isolated the meningococcus from the nasal secretion of a patient with cerebro-spinal meningitis and from that of a person in contact with such a patient. Weichselbaum and Ghon¹⁶ isolated the organism from the nasal secretion of one patient with cerebro-spinal meningitis and from that of three persons in contact with cerebro-spinal meningitis. Griffon and Gandy¹⁷ succeeded in isolating the meningococcus twice from one patient with cerebro-spinal meningitis, an interval of five days elapsing between the two times. Our own results along these lines will be found at the end of this paper.

A number of observers have succeeded in isolating the meningococcus from the blood by means of cultures (Solomon,¹⁸ Moller,¹⁹ Bettencourt and Franca,²⁰ Elser²¹). The last-named author examined 41 cases and succeeded in isolating the meningococcus from blood cultures in ten. He found that the occurrence of the organism in the blood was extremely irregular; sometimes the organism was present at the beginning of the illness; sometimes at the end. He found no relation between the presence of the organism in the blood and the severity of the disease.

Weichselbaum has recently published a case in which the meningococcus was found in the lesions of an ulcerative endocarditis. Drigalski,²¹ in one case, found it in the herpetic vesicles.

The above are practically all the observations on the occurrence of the meningococcus elsewhere than in the meninges of the brain and cord. An observation on the occurrence of the meningococcus in the dust of infected barracks can be dismissed as absolutely unreliable.

Viability of the Meningococcus—There are but few statements as to the viability of the meningococcus. Jaeger²² states that he observed that some meningococci which had been dried in pus were still viable after 127 days. On the other hand, Councilman says that so far as he was able to tell from its behavior in culture media and in the

tissues, "the meningococcus has a feeble vitality and would not be capable of leading a saprophytic existence. It must of course be remembered that in experiments to determine this viability, we cannot reproduce artificially all the conditions which the organisms find in nature." Bettencourt and França²⁰ made a number of experiments with cultures of meningococci. It was found that cultures dried on glass at various temperatures, 37 degrees, 20 degrees, 19 degrees C., had lost their vitality at the end of twenty-four hours. In many cases they were already dead at the end of three hours. Cultures exposed to direct sunlight where the thermometer registered 35 degrees to 37 degrees C. were dead at the end of two hours. Weichselbaum²³ also regards the meningococcus as having but feeble powers of resistance, and this opinion is shared by almost all the best workers.

Transmission of the Disease—Influence of Close Contact—That infection goes hand-in-hand with close contact between persons is shown, for example, by the following references: Frothingham²⁴ describes a small epidemic which broke out in *one* regiment of the Army of the Potomac. The soil was clay, weather mild and damp. The soldiers were crowded together, five to six men in a small tent. Conditions were such that remittent, intermittent and typhoid fever abounded. Nevertheless the cases of meningitis observed occurred only in *one* regiment, and this had by no means the worst quarters. Gifford²⁵ reports five cases of meningitis occurring in one family, one after another, within a week. He was unable to trace the cause of the outbreak. Hammer²⁶ in a discussion, remarked that in 1843 he had seen an epidemic in Mannheim, Germany. The garrison there consisted of 800 men, and the city contained 30,000 people. Yet no one was affected excepting the military. In two months about 50 cases had occurred, of which 11 were fatal. A few years previously a similar epidemic occurred in Strasbourg, also confined to the barracks. Corbin²⁷ describes an epidemic among the soldiers at Orleans in 1847-48. The townspeople were not affected. Jourdes²⁸ says the disease raged among the troops in Strasbourg for four months before it broke out among the townspeople. Here it occurred in 52 streets, but raged particularly in narrow, crooked streets where many people were crowded together. Mayne²⁹ observed epidemics which occurred in various workhouses

and hospitals. Gahlberg⁸⁰ remarks that certain streets are markedly affected. In one street there were 9 cases, in another four and in a third 6 cases. Those in the last-mentioned street were practically in neighboring houses. Most of his patients were robust. The cases extended from February to May. Ziemssen⁸¹ believes that conditions of soil are without any influence on the spread of the disease, and that the weather is also no factor. In general his cases belonged to those living in unhygienic surroundings. Only 5 (out of 42) belonged to the upper class. Most of the rest lived in poorly ventilated, damp dwellings and on the ground floor. But these conditions he says had existed a long time prior to the epidemic and can therefore be regarded only as auxiliary factors. Twice a house infection was observed; in one instance, to be sure, confined to two sisters. The other embraced four persons, who became sick in the course of two weeks. Three of these died. Jaeger⁸ says that an important factor in the spread of the disease is the rapid accumulation of people in cities and towns and a development of sanitary conditions of the dwellings which does not keep pace with this accumulation.

In the present epidemic in New York City both last year and this, the disease has affected chiefly the people living in the densely populated sections, the lower East Side, or "Ghetto," the lower West Side, and the "Little Italy" section in the vicinity of One Hundred and Tenth street and East river.

Influence of Age—So far as the influence of age on the prevalence of infection is concerned, the following statistics may be of interest. Hirsch² has collected the histories of 1,267 fatal cases occurring in Sweden from 1855 to 1860. Of these, 889 patients were under fifteen years, 328 between sixteen and forty years, and only 50 were over forty. In an epidemic in Bromberg, Germany, of 141 cases, 132 were between two and seven. In an epidemic in Thuringia, out of 180 cases, 160 were under twenty years. Leaving out of account the epidemics among soldiers, epidemics have also been reported in which the cases were mostly in persons between twenty and thirty years of age. Such was the case, for example, in the Italian epidemics in the forties, and in the epidemic in Montgomery, Alabama. In the latter, of 84 patients, 10 were under ten years of age; 23 between ten and twenty; 27 be-

tween twenty and thirty; 13 between thirty and forty; 12 over forty years of age.

In an epidemic of cerebro-spinal meningitis in Dantzic, Germany, there were 779 deaths. Of these, more than 25% were of children under one year; 88 per cent. were under ten years. In the epidemic in Cologne in 1885-1892 the majority of patients were between fifteen and twenty-five years.

We see from this that the disease at one time affects mostly infants, at other times older children, and at still other times adults. What the reason for this is does not seem at all clear.

Other Predisposing Factors—A number of writers lay stress on slight trauma as a predisposing cause and state that in many cases a history could be obtained of some injury to the head shortly before the onset of the disease. In our series of cases it was the exception to obtain such a history, although inquiries were always made. When it is remembered that children are constantly receiving all sorts of knocks it will not be surprising if now and then the disease will be found to have been preceded by some slight trauma. But that this is of any influence in determining the infection has nowhere been proved.

Some of the writers on meningitis mention the occurrence of a coryza or a sore throat just before the onset of the disease (Berdach³²).

Influence of Overexertion—Huebner³³ says that one of the factors predisposing to the disease is marked bodily, and perhaps, also, mental exertion. This was shown particularly in the military epidemics in France, where it was found that raw recruits unused to hard service were especially susceptible. That the latter statement, at least, is correct seems borne out by statistics from other sources. In the epidemics among the Wurtemberg troops in 1898, Jaeger³ found that out of 56 cases, 43 were among the troops of the first year of service, 11 of the second year, and 2 of the third year. But this does not show that it is due to overexertion. If that were the case we should expect far more cases in war than in times of peace, and yet, as we know, cerebro-spinal meningitis is not a disease of war. Jaeger also points out that the physical exertion required of the soldiers is carefully graded so that the recruits are only gradually put to hard work.

Virulence of the Meningococcus—It is possible that a given strain of meningococcus can in some way acquire an increased virulence and so start an epidemic. Since, however, this organism is so slightly virulent for ordinary test animals, we have no way of determining this except by studying the development of the various epidemics.

Studies on Animals—In the study of cerebro-spinal meningitis animal experiments have yielded practically no results of any importance owing to the fact that most animals are so little susceptible. Mice seem most so, but even these animals must be inoculated intraperitoneally; subcutaneous injections are usually without results. Guinea pigs are also somewhat susceptible, but not to as high a degree as mice. (Weichselbaum,²³ Bettencourt and França²⁰). Bettencourt and França injected three monkeys with cultures of meningococcus, one by trephining and two by spinal inoculation. They also rubbed a culture on a cotton swab on the nasal mucous membrane of a fourth monkey. None of these methods was followed by infection. Five goats were trephined and inoculated subdurally without effect excepting a slight fever. One goat was inoculated into the frontal sinus and another into the spinal canal, but without effect.

After irritating the nasal mucous membrane by means of ammonia we rubbed cultures of meningococci into the noses of several very young puppies, but did not succeed in producing an infection.

On the whole, practically all reliable authorities report negative results with animal tests.

Cerebro-spinal Meningitis in Other Animals—There are but scanty references to the simultaneous occurrence of cerebro-spinal meningitis among domestic animals and household pets. Magail²⁴ observed an epidemic of cerebro-spinal meningitis in Donera, Africa, in 1845, similar to one he had seen in France in 1837-42. The cases occurred suddenly among the soldiers in February. No cause could be found, but it was remarked that an epizootic had broken out among the barnyard fowl two weeks previously and raged among them for two months. Albrecht and Ghon¹⁵ in discussing this point say that the cerebro-spinal meningitis of horses has been said to be due to the meningococcus. The disease among horses (the so-called Borna'sche Krankheit) was very frequently observed in Saxony in 1894-96. Yet no cerebro-spinal menin-

gitis was observed among the people at that time. They think the causative agent of this disease to be entirely distinct from the meningococcus. The same view is held by Siedamgrotzky and Schlegel,³⁵ and by Johne.³⁶

We have made numerous inquiries among the veterinarians and stablemen in New York City regarding the presence of cerebro-spinal meningitis among horses. The answer has almost invariably been that no cases of the diseases have been observed for years. Two cases were mentioned as having occurred early in the season. One veterinarian, to be sure, stated that some three years ago he saw about 150 cases of the disease among some 8,000 horses belonging to a street railway company of this city. The statement could not be verified and may probably be dismissed as unreliable.

Insects as Carriers of the Disease—It has been suggested that the disease is carried from individual to individual by means of vermin or insects. So far as the writers have been able to discover, the literature contains no reference to this side of the subject; the indirect evidence appears to negative the assumption. This evidence consists in the geographical distribution of the disease, local distribution, season, class of people affected, etc. So far as the geographical distribution is concerned, we have already seen that this is very extensive. Locally, the disease has occurred in high and dry regions and in low, marshy ones; near the coast and far in the interior. The older observers were quite unanimous in believing that conditions of the soil and atmosphere were without influence on the development of the disease (Ziemssen³¹). Although the disease has in general been observed usually among the laboring classes, especially where the people were crowded together, it has often been observed to attack people in the best surroundings. Out of 42 cases reported by Ziemssen, 5 belonged to the upper classes. Holbrook³⁷ says that the cases observed occurred in the best and in the worst homes. Some patients were tenderly cared for, others neglected. They were equally affective.

The vermin theory of infection also presupposes the presence of the meningococcus in the peripheral blood of the patients. Although, to be sure, the meningococcus is frequently found therein, its presence does not appear to be at all constant throughout the disease (Elser³⁸). Even when present it does not appear to be so abundant that fleas or bedbugs

would be likely to ingest one very often. The length of time which sometimes elapses between primary and secondary cases of cerebro-spinal meningitis seems also to argue against this mode of infection. So far as mosquitoes are concerned, it will suffice to say that the disease is prevalent in this country at a season when there are practically no mosquitoes. It would be difficult, if not impossible, to reconcile all these facts with the assumption that an insect or parasite is liable to transport the disease.

Immunity and Susceptibility—So far as immunity is concerned, there is but little literature. North³⁹ gives one undoubted case in which there had been an attack twenty-five months previously. Another patient had the disease in August, 1808, and again in May, 1810. Herman and Kober⁴⁰ report a girl who had the disease in May, 1886, and died in the second epidemic the following year. Numerous instances are recorded in which several members of one family were affected. Friis⁴¹ observed house and family epidemics which affected two to five persons. Singer⁴² reports eight deaths in one family. Baxa⁴³ observed four cases in one house, three in another and two in a third. Brookes⁴⁴ also reports eight cases in the different branches of one family, in another there were five, and in still another family, four cases. Ziemssen³¹ reports the case of two sisters who were affected. Gifford²⁵ reports five cases in one family, one after another within a week.

In the series of cases studied by us, in two instances the disease had occurred in the same family a year ago (cases 19 and 52). It is possible that cases 11, 13, 37 and 54 may also be instances of special susceptibility, for in these all the children of the family developed the disease. The same may apply to case 54, in which four out of five children were attacked. The presence of a special susceptibility is perhaps also indicated by the fact that in most of the families investigated, only one or two children out of several were affected. On analyzing the histories of 45 families in which a number of children is noted, we find that these families had a total of 210 children. Of these 210, 63 became infected, while 147, who were probably equally exposed to infection, escaped. Since our own investigations have shown that many healthy persons in contact with cases of cerebro-spinal meningitis may harbor meningococci in their noses, it would appear that these persons were more or

less immune to such infection. This immunity, of course, can be conceived as being either local (nose or meninges) or general.

Communicability—There seems no doubt among the majority of observers as to the transportability of the disease, but there is no unanimity as to how this is effected. W. H. Draper⁴⁵ observed an epidemic in Carbondale, Pa. He found that the disease had begun in two places; 1st, where there had been a camp of soldiers, and 2d, where some tramps had carried away things belonging to dead soldiers. Hirsch² expresses himself as follows: "Connecting with the infective nature of epidemic meningitis is the question of its communicability or contagiousness. Most observers have answered it quite decidedly in the negative on the ground of their experience, that those who have come into close and continuous contact with the sick, such as medical attendants and nurses, have been very rarely attacked, and that patients suffering from it had been admitted into the wards of hospitals without any extension of the disease to the other patients ever taking place. On the other side, there are facts that tell in favor of communicability, the most notable of these being the observations made in the epidemic of 1837-40. In these epidemic the disease would seem to have been transported by infected troops from place to place, sometimes even to distant garrisons, where it did not confine itself to the division of troops originally, but spread in epidemic form to several other regiments. Baudin⁴⁶ thinks the disease contagious and has collected the following interesting points:

In 1841 a battalion of troops marched from Pont Saint Esprit, where the disease raged, to Marseilles, which was not yet infected. Shortly afterward two other battalions of the same regiment returned from Algiers and were distributed among the barracks with the first battalion. A little later a case of meningitis broke out among the second battalion.

In 1847 a division of troops, who had suffered much from meningitis at Avignon, was sent to Nimes. No further cases occurred here among them. But some healthy troops which had arrived from Africa also arrived at Nimes, and among these, cases of meningitis broke out soon afterward.

In Orleans it was twice observed that soldiers sleeping next one another were affected. The two cases outside of the garrison were the mistress of one of the soldiers and her child. In St. Etienne, in

1840, two soldiers who shared one bed were taken sick, one within forty-eight hours of the other. In one of the barracks a soldier returning from guard duty lay down in a bed from which another who had been taken ill with meningitis had just been removed. The second soldier became infected and died. In Aignes-Mortes, out of the people living in one house five were affected, of whom four died. In Strasbourg, among others, there were affected two surgeons, one clinician, five military nurses, seven children of soldiers and several workmen employed about the barracks.

While the disease raged in Strasbourg a regiment from there was sent to Schledtstadt on January 21, 1841. On the twenty-ninth of that month the first case appeared in that town, the child of an innkeeper near the barracks, the inn being much frequented by the soldiers. On February 6, two children, the daughters of the butcher supplying the garrison with meat, were affected. Hirsch² describes a remarkable outbreak in Algiers in 1840, a season when the disease was more than usually prevalent among the troops in France. Algiers is the only spot on African soil where the malady has ever been seen; it is in intimate relations with France, and importation of the disease is made all the more credible by the fact that its first appearance there was among the French troops, with subsequent extension to the civil residents. Horner⁴⁷ suggests that in the epidemic in the hospitals of Washington, D. C., in 1864-5 the disease had been introduced from the seat of the war. Broussais⁴⁸ has come to quite different conclusions regarding the spread of the disease. He says that the epidemic affected chiefly the military. It began at two points in France in 1837, namely, Bayonne and Narbonne. From these it spread in somewhat radiate fashion. The disease did not seem to be due to a transportation of the virus through the marching regiments, for the disease crossed the Italian frontier and raged in Italy in 1840-41. In no instance could direct contagion be demonstrated. Out of 1,041 cases there were 592 deaths (1:1.75). Brooks⁴⁴ investigated the history of 112 cases and says: "So far as contagiousness is concerned, the answer from the different reporters was universally 'No.'" The only evidence that supported the theory was that in a few instances more than one case occurred in the same family. The evidence becomes less valuable,

however, when we find that these cases occurred almost simultaneously with each other, or were so far separated as not to show any relationship with each other. One of the very best studies on the spread of the disease was made by Peterson,⁴⁹ who investigated the histories of the cases occurring in Berlin in 1895-6. By extremely careful work he was able to connect a series of 23 cases one with another, thus showing a high degree of communicability of the disease.

Original Investigations—The clinical data on which this study is based were limited to cases occurring two or more in one house in the period from January 1, 1905, to June 1, 1905. This limitation was maintained because it was felt that their careful analysis would be more apt to throw light on the mode of transmission than would an analysis of the other cases.

The bacteriological study, on the other hand, while it included some of the cases of the clinical series, sought especially to obtain data on the occurrence of the meningococcus in the nose. For this reason it embraced an extensive series of individuals, including not only cases of cerebro-spinal meningitis, but also persons apparently perfectly well.

Clinical Study—The record of cases of cerebro-spinal meningitis, which is under the direction of Dr. Billings, is kept on the card catalogue system and arranged by streets and numbers. It is therefore a comparatively easy matter to run over the cards and note the names and addresses where several cases have been reported from one house. The cards from January 1st to June 1st numbered just about 1,500, and among these, 88 instances (representing 200 cases) of multiple cases were discovered. Lack of time prevented the investigation of all these 88 instances, but the following results, obtained by a careful study of 58 instances, would in all probability apply to the remainder.

1. At 488 Eleventh avenue, Johnny, ten years old, died in March, after an illness of one week. He was not sent to the hospital. On May 2, his brother, Tommy C., aged eight years, developed the disease, dying on the following day. There are three other children in the family. Besides these cases the disease appeared in *another family* in this house. Ed. M., five years old, was sick from March 17, about one week after J.'s onset. There are two other children in the M. home.

No further contact can be discovered except that the children played together in the street.

2. Michael K., two years old, of 64 Amsterdam avenue, became ill with cerebro-spinal meningitis April 24, and died May 8. He was not taken to a hospital. On May 3 his little sister Agnes, aged four years, became ill with the disease. She has since recovered. There are three other children in the home. Agnes did not sleep with Michael.

3. Nellie L., aged three years, of 317 East Thirty-eighth street, became ill with cerebro-spinal meningitis on March 25. Two days later, Mary, aged eleven years, developed the disease and died in five days. Nellie recovered. There are six children in the family, which occupies four rooms. There was another case of cerebro-spinal meningitis in the house (but the people have moved and it is impossible to get data about it). It was reported April 27 and was a child one year old.

4. Celia M., in 425 West Thirty-first street, 9½ years old, was taken ill with cerebro-spinal meningitis on March 3, and died March 4. Five other children at the time, the family living in four rooms. Previous to the illness all these children slept in one bed. On April 5 one of these developed the disease and died April 6. There are no cats or dogs. There may be bugs.

5. Michele T., aged five years, of 161 West Twenty-seventh street, died of cerebro-spinal meningitis on April 1, after an illness of twenty-four hours. There were five other children, the family living in two rooms on the fourth floor. On April 19, R. F., aged 11½ years, living on the second floor, developed the disease and was taken to Bellevue the same day. There is very much intimate intercourse between these two families, children going into each other's homes all the time. The F. family also have two rooms, fairly clean. Two other children still left. These were not affected.

6. Katy S., aged sixteen months, living at 347 East Seventy-third street, died of cerebro-spinal meningitis on March 22, after an illness of one day. There is one other child. Among those who came to the funeral was Mr. P., living on the same floor. None of the P. children or Mrs. P. attended. Peter P., aged three, developed the disease and died on March 30. There are three other children in the family, which lives in three rooms; mother and father go out to work, the smaller

children being taken care of by a family across the street. Interval between cases, six days. There is another case of cerebro-spinal meningitis on the ground floor of the same house. Mary S., aged thirteen years, the daughter of a small shopkeeper (furniture), was taken ill February 16; she died March 19. Typical history. Two other children, ten and twelve years. No direct contact can be discovered between this family and either of the preceding.

7. Elizabeth S., 402 East Sixty-fifth street, $2\frac{1}{2}$ years old, suddenly developed cerebro-spinal meningitis on the night of March 29. The child did not play out in the street with other children, but was taken out daily by her mother. The day preceding her illness she had played on the roof in some sand. Her mother was hanging out the wash. Playing in the same heap of sand was a Johanna St., age $3\frac{1}{2}$ years, living in the same house (model tenement). Johanna was taken sick two days after Elizabeth and died April 8. The St.'s keep one dog. Both families have excellent clean apartments, plenty of light and air.

8. At — Hospital a chronic rheumatic who had not been outside the hospital for many months suddenly developed cerebro-spinal meningitis. Three weeks before this there had been two cases of the disease on the same hall. All of the patients, including the rheumatic, had private rooms. Connection between these cases is apparently through the nurse, he having attended all three cases.

9. Bartholomew M., age twenty-three years, died of cerebro-spinal meningitis on January 31 after an illness of two days. He was removed to the hospital the day he was taken sick. The young man boarded with Mrs. Hart, the housekeeper of the building. This woman sweeps and cleans the halls, etc., daily, and sets out the ashes, but does not come in contact with the tenants any more than an agent collecting the rents. On March 20, Bridget L., aged twenty-one years, one of the tenants living on the next floor above, developed the disease. She has since recovered. There are two children in the L. family; none in the H. No cats in either home.

10. Joe F., aged four years, of 593 Greenwich street, rear house, was taken sick with cerebro-spinal meningitis on March 7 and died March 9. The family consists of the parents and two other children. They occupy two rooms. On March 11, John C., aged five years, a

playmate of Joe F., developed the disease and died March 14. Two other children in the family. Two rooms. Filthy; no cats. Joe's most intimate chum was another boy named Buster. This boy, however, was not attacked by the disease.

11. Isaac C., 174 Clinton street, aged ten years, developed cerebro-spinal meningitis about three months ago on a Saturday afternoon. That same evening his brother Moe, aged four years, developed the disease, dying within twenty-four hours. On Monday two others. Thereupon all three were sent to Gouverneur Hospital, where they finally recovered. When Isaac and Moe became sick on Saturday, the parents sent Louis and Philip out of the house to some relatives on Sunday. Nevertheless they developed the disease in the latter's house the next day. In this house these two children slept together with the other children in that family. None of the latter, however, were affected.

12. Joe N., at 142 Tenth avenue, aged three years, died of cerebro-spinal meningitis about April 7, after an illness of a few days. One month later May H., aged three years, living on the next floor above, developed the disease. The N.'s have one other child; H.'s have four other children. The children of both families play together, but otherwise there is no intimate relation between the two families. Meningococci were isolated from the nose of Joe on the second day of the disease.

13. Laura G., at 458 Eleventh avenue, 3½ years old, and James G., five years old, developed cerebro-spinal meningitis the same day, May 19. Laura died the following day, James was sent to Bellevue Hospital. On May 24, Richard, six years old, developed the disease. There are no other children. They all slept together. Four rooms, rather dirty, very dark.

14. Rosie S., aged nine years, at 172 Rivington street, developed cerebro-spinal meningitis April 18, and died April 23. A week after her death, Joe, her brother, aged five years, developed the disease. He is now convalescent at Gouverneur Hospital. There are three other children in the family, which occupies three rooms. Meningococci were isolated from the nose of Joe on the fifth day of the disease.

15. Louis M., at 233 East Ninety-seventh street, aged nine weeks, died of cerebro-spinal meningitis after an illness of one week. He was kept at home during the illness. His sister Molly also developed the disease. After remaining at home for eight days she was sent to the Presbyterian Hospital, where she died after a total illness of about one month. Prior to her removal to the hospital, and while she was already sick with cerebro-spinal meningitis, Molly was allowed to sleep with the other children. Nine days after this removal to the hospital, another sister, Cecilia, aged fourteen years, developed the disease. She was also sent to the hospital and is now convalescent. Beside the above there are three other children in the family. The rooms are fairly clean; there is one cat; also rats and mice.

16. Rachel K., at 107 Forsyth street, top floor, aged seven years, developed cerebro-spinal meningitis about March 3, and died in three weeks at Beth Israel Hospital. She was at home four days before her removal there. The family occupies five rooms fairly clean. There are four other children. About one month later Elizabeth E., one year old, living on the same floor of this house, developed the disease. She died May 6; there are four other children in this family. There is considerable friendly intercourse, especially between the children of the two families. No cats in either home.

17. Willie S., twelve years old, of 117 Norfolk street, developed cerebro-spinal meningitis February 22, and was sent to the hospital three days later. He is now well, but not yet out of the hospital. There are seven other children, and they live with their parents in three rooms. On May 15 Lena H., aged eight years, developed the disease, and died May 16. There are five other children in the H. family. There is considerable intercourse between the children of these families, and some of them are in the same class in school.

18. Esther W., aged ten years, of 96 St. Mark's place, felt sick with headache May 15, and became violently ill of cerebro-spinal meningitis on the morning of May 16. On the night of May 16 her playmate, Bella, aged 6½ years, developed the disease and was taken to the hospital the next day. There are several other children in each family. Beside contact through the children, it was found that Mrs. F. visited the W. child the morning she was sick in bed, May 16.

19. Esther S., then living at 19 Allen street, had cerebro-spinal meningitis from June 1, 1904, and died in about two weeks. Her sister, Rebecca, developed the disease about June 11, 1904, and was taken to Gouverneur Hospital June 12. Discharged cured September 14, 1904. Since then the family has moved several times. They now reside at 4 First avenue. On May 19, 1905, Theresa S., aged four years, developed the disease and is now in Gouverneur Hospital. There are now four other children at home. Three rooms, clean and light.

20. Sarah G., at 118 Delancey street, seven years old, developed the disease on January 13, and died January 22. Two weeks after her death her sister Rachel, eleven years old, developed the disease. She is still sick. The family have one other child beside these. They occupy two rooms, quite dirty. No dogs or cats.

21a. Louis Z., seven years old, of 85 Willet street, third floor front, became ill with cerebro-spinal meningitis April 30, and was taken to Gouverneur Hospital the same day. Both he and his brother and sisters (ranging in age from one to eight years) often played with the children of a family, C., living on the floor below. On May 20 the disease broke out in the C. family (see next case). Meningococci were isolated from Louis Z.'s nose on the third day of the disease.

21b. Charles C., of 85 Willet street, aged nine years, developed cerebro-spinal meningitis on May 20, and was taken to Gouverneur Hospital May 23. On May 22 his little brother, Tobias, four years old, developed the disease. Both are still sick. Beside these two children the C. family has one other small child and several older children who go to work. They occupy three rooms.

22. Jennie D'A., aged $2\frac{1}{2}$ years, of 316 East Eleventh street, second flight, was taken sick with cerebro-spinal meningitis March 2, and died March 3. On March 7, Rosie, aged $3\frac{1}{3}$ years, a sister of Jennie, developed the disease and died within twelve hours. The children did not sleep together. No previous case known. Family occupies three rooms and consisted of the parents and six children. There are no bedbugs; rooms clean.

23. Nicholas D., $3\frac{1}{2}$ years old, of 2134 First avenue, taken sick with cerebro-spinal meningitis February 22. On March 1 his brother C., aged four months, who had slept in the same bed with him, de-

veloped the disease and died April 21. There are six other children, mostly older. Family has three rooms, moderately clean. No cats.

24. Camella G., sixteen years old, 2076 First avenue, third flight, was taken sick with cerebro-spinal meningitis March 7, and died May 3. There are three other children in the family. While Camella was sick, among others who came to visit was Mrs. S., who lived on the floor above. On March 28, Maria S., daughter of the latter, developed the disease; she has now completely recovered. In addition to contact through Mrs. S., there was much contact between the other children of both families, who constantly played together.

25. Eddie S., of 1699 Third avenue (eleven years old), had cerebro-spinal meningitis in December and died just before Christmas. Among his chums was a boy named C., living across the street (1650 Third avenue). In addition to this, the other C. and S. children were very good friends and visited each other's homes frequently. On February 24, Theresa C. (sister of the C. boy), aged $5\frac{1}{2}$ years, developed the disease and died February 25. On February 25 her sister Kate, $2\frac{1}{2}$ years old, developed cerebro-spinal meningitis and is now in the hospital. On February 27 Maggie, aged seven years, developed the disease. She recovered, but is deaf. There are four children still left. Interval between S. and C. cases, two months. Intervals between the three cases occurring in the C. family were one and two days.

26. Pauline P., of 3 Hester street, aged eleven years had cerebro-spinal meningitis March 18, and died the same day. Besides the parents there are four other children, also one woman with a baby. All these live in three rooms. On the same floor in the front of the house lives a family, F., who are very friendly with the P. family. The children are always in and out of the apartments. On March 23, Mary F. developed the disease and was taken to Gouverneur Hospital next day. She has now recovered. There are three children and the parents in the F. household.

27. Sadie S., at 78 East One Hundred and Thirteenth street, eleven years old, became sick with cerebro-spinal meningitis March 10 (Harlem Hospital, then Bellevue; recovery). Her brother Max also developed the disease twelve hours later. Recovered. Mrs. S. says the girl complained of fever and chilly sensations for two days prior to

onset. She insisted, however, on going to school. During this prodromal stage there were no headaches, no vomiting.

28. Liboria C., ten years, Joan C., eight years, and Ernesto C., seven years, of 431 East One Hundred and Fifteenth street, all developed cerebro-spinal meningitis on the same day about two months ago. Ernesto died. No trace can be found of source of infection.

29. Francesca M., of 195 Elizabeth street, was taken sick with cerebro-spinal meningitis about March 10. She ran a typical course for twenty-two days. About April 5 she had a relapse and was taken to Bellevue April 27. On April 17 Joe M., seven years old, a brother of the preceding, suddenly developed the disease. He had not slept with the patient. The grandmother of the children suddenly became sick with cerebro-spinal meningitis on April 22, and died in Bellevue April 29. She had not slept in the same room with either patient. The family occupied three rooms on the top floor of a dirty tenement. No cats or dogs. Bugs probably present. Meningococci were isolated from the nose of Joe on the twelfth day of the disease and from the nose of the grandmother on the fourth day of the disease.

30. Katy K., five years old, 224 West Sixty-seventh street, died of cerebro-spinal meningitis about five weeks ago after an illness of one week. One week after the onset of the disease, Jennie L., also five years old, and living on the floor below, developed the disease. No direct connection can be made out and no good history obtained. Neither family at home. It is very likely, however, that the children would play together, as they are of the same age.

31. Kitty T., aged three years, of 308 West One Hundred and Thirty-fifth street, died of cerebro-spinal meningitis about one month ago after an illness of three weeks. A week after she was taken sick the baby, Tony, one year old, developed the disease and died in ten days. There are no cats or dogs about; there may be bugs.

32. Francis C., aged two years, of 20 East One Hundred and Thirty-second street, became sick with cerebro-spinal meningitis on February 16 and died two days later. His father, a carpenter by trade, did not feel quite well on February 17 and became very sick on February 18, in the morning. He died February 22. There are two other

children and the mother still living. They have returned to Carbondale, Pa. Family occupied four rooms on top floor, all light; no cats or dogs.

33. Gertrude F., three years old, 124 East One Hundred and Eighteenth street, became sick with cerebro-spinal meningitis March 27, and died within twenty-four hours. Four days after her death her sister Flora, one year old, developed the disease, and died May 12. There are two other children. Flora was taken to the hospital at once. Family occupied four rooms, fairly good condition. Had no pets.

34. Lester G., of 235 East One Hundred and Second street, top floor, aged $2\frac{1}{2}$ years, was taken sick with cerebro-spinal meningitis on March 25, and died May 6. There were three other children in the family, but they did not sleep with patient. The eight-year-old child of the janitress, Mrs. L., living on the ground floor, developed the disease, and died in a few days. There is but little intercourse between the families, and the children do not play together (Mrs. L., has three children). Mrs. L. visited the G. family once at the beginning of the illness. It was about a month, she says, before the illness of her own child. The G. family did keep a cat; there are a few bedbugs; no roaches. No cats or vermin (?) in the other house.

35. Henry W., aged thirteen years, 206 Broome street, was taken ill with cerebro-spinal meningitis suddenly and taken to the hospital next day. Eight days later his brother Aaron, aged six years, developed the disease. Both patients recovered. There are five other children in the house and the family occupies three rooms. There are no bugs (?), but two cats. Interval, eight days.

36. Kathleen S., 115 East One Hundred and Second street, aged sixteen months, was suddenly taken ill with cerebro-spinal meningitis on March 12, and died on March 14. Five weeks later her brother Daniel, $3\frac{1}{4}$ years, developed the disease, dying two days later. There are no other children in the family. Both patients slept with their parents in one large bed, and they slept there even while sick. Interval between cases, five weeks. There are no cats or dogs and no vermin. The rooms are moderately clean.

37. Baby H., eleven months old, at 208 East Ninety-sixth street, top floor, developed cerebro-spinal meningitis about April 2 (is still

alive). Besides the parents there are two other children. Across the hall on the same floor lived the family O., with three children, who visited in the H. apartment constantly. On April 6 or 7, Mrs. O. says her daughter, Wilhelmina, aged eight years, was in the H. apartments and hung about Mrs. H., who was carrying her sick child about. On April 8, Wilhelmina became sick with typical symptoms of cerebro-spinal meningitis, and died April 11. Both apartments are quite clean, large, well-lighted and well-ventilated. No bugs; no pets. Interval between onset of two cases, six days.

38. At 308 East One Hundred and Sixth street, third floor, rear, there lived the families C. (man, wife and two children), and Ch. (man, wife and baby). Altogether they have three rooms (history obtained from neighbor as people were not at home). One of the babies became ill with cerebro-spinal meningitis and died. The second baby became ill two days after the first. Apartments in the house very dirty. No cats. Interval, two days.

39. John R., fifty-three years old, of 407 East Sixtieth street, died of cerebro-spinal meningitis on April 3, after an illness of four days. There still remain of the family the wife and one son. Next door to R. lives the family P., relatives of the former. During the illness and at the funeral, Mrs. P. was in the R. home frequently. Some of the children were there also. Ten days after the funeral Nellie P., thirteen years old, developed cerebro-spinal meningitis. She is now recovering. The P. family occupy four rooms. Besides the mother there are six children.

40. Rosie B., of 231 Eldridge street, was a patient at Gouverneur Hospital suffering from cerebro-spinal meningitis. She ran an atypical course. Had slight tenderness at the back of the neck. Some difficulty in walking during convalescence. There was no eruption. Temperature dropped by crisis. No lumbar puncture was made. Max B., brother of the preceding, was taken sick about three weeks later and also ran a very atypical course. No rigidity, no Kernig sign. Had a peculiar macular bright red eruption which was regarded as possibly scarlet. A diagnostician from the Department of Health, however, pronounced it not scarlet. The spots were very close together and all over body. They resembled typhoid spots. Temper-

ture dropped about the tenth and eleventh day. Spinal puncture was made three days after that (for diagnosis). It showed very many meningococci (Dr. Goodwin).

41. Theresa S., at 329 West Thirty-ninth street, was taken ill with cerebro-spinal meningitis on March 24, and died within twenty-four hours. Her sister, Rosie, who slept in another bed, developed the disease twelve hours after Theresa. She died in three days. There are five other children in the family.

42. Rosa P., aged six years, living at 222 West Thirtieth street, was taken ill with cerebro-spinal meningitis some time in January, dying in twenty-four hours. Six days later her mother developed the disease and died in forty-eight hours. The mother had about ten children, some of whom were married. Five unmarried children, however, still lived with her. Four rooms, quite clean, top floor back. Plenty of light. There is one cat. Bugs (?).

43. Evelyn D., five years old, of 147 West Sixtieth street, developed cerebro-spinal meningitis after an outing in the park. Her mother says the girl fell and struck her head. But there was no loss of consciousness and child appeared to be perfectly normal after the accident until about twelve hours after. During the night she was suddenly attacked with vomiting, headache and fever. Her physician was positive of the diagnosis cerebro-spinal meningitis. She died on April 10. There were no other children in the family. Just one week later a chum of Evelyn, living one floor lower in the same house, Annie McC., eight years old, developed cerebro-spinal meningitis and died in two days. Mrs. McC. says that during Evelyn's illness and at the funeral, her children had all been up to see Evelyn (there were five McC. children, all older than Annie).

44a. Julia C., of 406 West Forty-second street, six years old, taken sick with cerebro-spinal meningitis on May 3. Three days later her sister, E., three years old, developed the disease. Prior to Julia's illness she and Elizabeth slept in the same room, but not in one bed, Julia sharing a bed with her little brother, John. There is one cat in the house. No bugs; plenty of room. Clean (Elizabeth had been subject to repeated attacks of convulsions for a couple of years. Mother thinks perhaps cerebro-spinal meningitis diagnosis not true). Both

children were taken to Bellevue after Elizabeth was taken ill, and both died there.

44b. Julia C. attended a kindergarten in Forty-second street and had told her mother of a girl being sick there with cerebro-spinal meningitis. This case was looked up and proved to be Juliet B., aged four years, of 448 West Forty-second street. She was taken ill about February 6 and stayed at home until April 25. Then she returned to school for three or four days. Diagnosis of her illness not conclusive, though she had severe headache, was very drowsy and kept to her bed. She lost considerable flesh and was not able to walk well when she recovered. Her physician pronounced it cerebro-spinal meningitis.

45a. Caroline J., of 417 West Fortieth street, second floor front, six years old, was sick early in February with cerebro-spinal meningitis and died at the end of that month. There are two other children in the home (aged eight and nine years). The home consists of three rooms. There are no dogs, no cats. Children play all over, the mother going out to work by the day. On the ground floor of this house is the shoe-maker shop of R. Here a boy, Paul R., aged two years, developed cerebro-spinal meningitis on April 10, was taken to the hospital on April 23, and died there April 28. There are two other children older in the house. Besides the shop the family have two living rooms only. There are no cats or dogs. Apartment moderately clean. It is not possible to connect this case directly with the preceding, though of course the children may have come in contact with each other.

45b. The J. girl attended a kindergarten connected with a small public school (eight small rooms). Directly next door to this school, one of the pupils, S. by name, died of cerebro-spinal meningitis December 21. He was in the class ahead of the kindergarten, and of course may have played with the J. girl. No positive connection between the cases can be established.

46. Blanche M. was ill with cerebro-spinal meningitis in 266 West Thirty-ninth street, and died. One of her chums was Kitty A., a classmate from school. Mrs. A. was afraid Kitty might contract the disease, so took her to the country after Blanche's death. Mrs. M. has recently heard of Kitty's death from the same disease.

47. No. 443 East Thirteenth street, a four-story tenement with a rear house. The following cases have occurred there, but it is not possible to find the connection between them: About six weeks ago a boy living on the second floor front, east, developed cerebro-spinal meningitis, dying in twenty-four hours. There is one other child, seven years old, but not affected (cannot find out name of the family). About two weeks later, James C., living on the top floor front, developed the disease. He is still alive (May 13). There are no other children in this family. Parents say James did not play with children of the first family. Two weeks after the onset of C.'s illness the disease appeared on the ground floor in the family of V., who keeps a butcher shop. His daughter, Annie, became ill and died in four days. There are two other children, but they were not affected. No cats; no dogs. Two days after death of Annie V., a baby, R., living in the rear house, second floor, was taken ill with cerebro-spinal meningitis. She died (?). No other children in the R. family. No intercourse can be made out between the various cases. A child died of cerebro-spinal meningitis in the family of L., 445 Thirteenth street, who keeps a saloon on the ground floor. His child was nine years old and died after an illness of five days. Typical symptoms. This was about six months ago.

48. Mrs. S., of 501 East Seventy-sixth street (tenement) had a baby sick with cerebro-spinal meningitis from February 10, 1905. The child died March 7. Among those who came to express their condolences was Mrs. Schn., living on the same floor, who stayed but a minute or two. On March 18 the latter's thirteen-year-old son developed the disease and died two days later. No other contact can be discovered. No other children. Each family occupies three rooms. Interval between cases, eleven days.

49. Mrs. W., of 871 First avenue (tenement) had a child die of cerebro-spinal meningitis on February 24, after an illness of three days. On March 17 the nine-months-old baby of Mrs. J., the janitress, developed the disease and died in ten days. Mrs. J. lives two floors below W., and although she renders no real janitor service (no dumb-waiter), visits the tenants to collect the rent. Interval between cases, twenty-two days. It may be remarkable in passing, that Mrs. W. lost

a child of cerebro-spinal meningitis in March last year, eleven months ago. At that time she lived in this same house.

50. In 75 Baxter street, an Italian family, B., living on the fifth floor of a large tenement, had a baby aged eight months sick with cerebro-spinal meningitis. Eleven days after the onset the baby's brother, eight years old, developed the disease and died in two days. On the seventh floor of this tenement lives another Italian family, C. Their baby, one year old, was taken sick with cerebro-spinal meningitis about three weeks after the B. boy had died and while the B. baby was sick in the house. The C. baby died in a week. There are other children in both families, and they all play together.

51. In No. 13 Little West Twelfth street, Mrs. F. had her four children all stricken with cerebro-spinal meningitis within a few days. All of them were at once sent to St. Mary's Hospital. Two of them died, one March 9, the other March 11. On the death of her two children Mrs. F. visited a family D. living on the floor below, and cried a great deal as she related her story to Mrs. D. Five weeks after the last child had been taken to the hospital, and about the same time since Mrs. F.'s visit just mentioned, the D. baby developed cerebro-spinal meningitis. The following day the five-year-old boy was taken sick, and three days later two more developed the disease. Three of these have died. There is considerable intercourse between the families. At the time these facts were elicited, four days after the last of Mrs. D.'s children had been sent to the hospital, nasal swabs were taken from both Mr. and Mrs. D. Both were found loaded with meningococci.

52. At 517 West Forty-sixth street, a young man, A., developed cerebro-spinal meningitis suddenly after playing ball in Central Park. He was treated at home for four days, and then sent to Roosevelt Hospital, where he died in two days. During his stay at home he had been nursed by his mother, Mrs. R. She developed the disease five days after the boy's removal to the hospital and died in two days. Interval between cases, five days.

53. At 428 West Thirty-seventh street, Kate D. developed cerebro-spinal meningitis on March 30 and was removed to the hospital the

next day. Her baby brother, thirteen months old, was taken sick eleven days after this and died in one day. Interval between cases, eleven days.

54. At 158 West Twenty-eighth street, a boy $2\frac{1}{2}$ years old, the child of a fruit pedlar, M., developed cerebro-spinal meningitis. He was sick only one day and died March 29. Five days later two other children were taken sick. One of these has completely recovered; the other is still living (iv-18) in the hospital. Interval between first and second case, five days. A nasal swab taken from Mrs. M., fourteen days after the children were sent to the hospital, showed very many meningococci. Swabs made from the other children and from the convalescent case were negative.

55. In 86 Horatio street, Mary, aged six years, was taken ill with cerebro-spinal meningitis on Christmas eve. She was sick for about two months and recovered, although deafness resulted. A week after the onset, the girl's aunt, Elizabeth F., who lived in the same apartment and had nursed the girl, developed the disease and died after an illness of one week. Two weeks after the funeral of the aunt, and while the first case was still in the house, a little baby sister of this first patient became ill. She died after an illness of five weeks, during which time she had many convulsions and high fever. (The attending physicians said she died of convulsions due to teething and that she did not have cerebro-spinal meningitis.)

56. At 131 Thompson street, a boy, A., eleven years old, developed cerebro-spinal meningitis and died in twenty-four hours. Two months after this two other children living in this apartment, one a brother, A., aged six, the other a cousin, G. (aged four years), of the first patient, became ill. Both died after an illness of three or four days. Interval between the occurrence of the cases, two months.

57. At 515 East Twelfth street, John E., two years old, was taken sick the beginning of March. Three weeks later, while the first patient was still alive in the house, his brother (eight months) Andrew developed the disease. Both died about April 7.

58. At 307 East Forty-fifth street an Italian family, S., have a girl aged $3\frac{1}{2}$ years, sick for the past two months with cerebro-spinal meningitis. A month after the onset the two-year-old boy in the family,

P., living on the floor above, developed the disease. Both patients still sick. There are other children in both families, but these are not ill.

The 58 instances represent 144 cases, as follows:

39 instances with 2 cases to a house.....	78 cases.
15 instances with 3 cases to a house.....	45 "
2 instances with 4 cases to a house.....	8 "
1 instance with 5 cases to a house.....	5 "
1 instance with 8 cases to a house.....	8 "

In 34 of these 58 instances there was an interval of varying length between the death or removal of the first case and the onset of the disease in the subsequent case or cases. The length of the interval may be tabulated as follows:

- In 14 instances it was from 1 to 7 days.*
- In 5 instances it was from 1 to 2 weeks.
- In 4 instances it was from 2 to 3 weeks.
- In 3 instances it was from 3 to 4 weeks.
- In 2 instances it was from 4 to 5 weeks.
- In 3 instances it was from 5 to 6 weeks.
- In 2 instances it was from 7 to 8 weeks.
- In 1 instance it was 3 months.

The incubation period of cerebro-spinal meningitis is usually considered to be about four days. If, therefore, we subtract from the above 34 cases the nine which developed within four days, we have 25 cases in which the question arises as to where the infecting organism was harbored in the meantime.

This question is answered, in part at least, by the results obtained by the bacteriological examination of nasal swabs from the parents of some of the children sick with cerebro-spinal meningitis. In three cases of the present series a large number of meningococci were obtained in cultures from these swabs (See cases No. 51 and 54). That the organisms are really meningococci will be seen by a study of Dr. Goodwin's paper on the bacteriology of the meningococcus. It may be added that in one of these cases the organism was obtained fourteen days after the last contact with patient, and in the other four days after such contact. Meningococci were also isolated from the nose of

*In nine of these 14 it was four days or less.

five cases suffering from cerebro-spinal meningitis. (Cases 12, 14, 21a and 29.)

In 18 of the 58 instances studied the second case developed while the first was still in the house. The interval between the onsets in these instances was as follows:

In 3 cases it was 1 day.

In 4 cases it was 2 days.

In 1 case it was 3 days.

In 1 case it was 5 days.

In 1 case it was 6 days.

In 4 cases it was 7 days.

In 1 case it was 9 days.

In 1 case it was 11 days.

In 1 case it was 21 days.

In 1 case it was 30 days.

In three of the instances encountered the disease occurred in the families of janitors and seems to have been communicated to or by them in the ordinary intercourse with the tenants. These are cases Nos. 9, 34, 39.

In two instances the disease appears to have been contracted in school. Cases no 44b, 45b.

In four instances mothers or other relatives taking care of children suffering from cerebro-spinal meningitis developed the disease. Cases 29, 42, 52, 55.

Period of Incubation—Case 7 of this series is of considerable interest, for it throws some light on the period of incubation. In this case two children developed the disease within two days of each other, and the only contact between them had occurred a day previous to the onset in one of them. If both children became infected from a common source the incubation period is *one* day for one child and *two* days for the other. If, however, the second child was infected from the first, the incubation period is exactly three days. The latter assumption seems the more likely, for this period of incubation agrees more closely with other cases in the literature. Thus Richter²⁰ gives the case of a woman who visited a house in which two children were sick with cerebro-spinal meningitis. She stayed in the house one day and then visited her uncle. Four days later she became ill with cere-

bro-spinal meningitis. During the last few days of her illness she was visited by a young man, who in turn developed the disease in a mild form four days afterwards. At the end of five days he was able to return to business. Five days after his return one of his fellow clerks developed the disease.

Dwelling Infections—There are practically no references in the literature to “dwelling or house infections,” such as are seen in tuberculosis. As a rule, when more than one case is mentioned as occurring in a house or building the infection appears to have been a more direct one. In the present investigation we have not met with a single instance of true “house infection.” In view of the feeble vitality of the meningococcus, this is about what was to be expected.

Summary To summarize our knowledge concerning the epidemiology of cerebro-spinal meningitis and the mode of infection we may say:

1. The disease has occurred in several large epidemics during the past century; sporadic cases are met with in the periods between these epidemics and constitute the link between the epidemics.

2. We do not know the circumstances giving rise to these epidemic outbreaks.

3. The epidemic form of cerebro-spinal meningitis is almost invariably associated with the meningococcus of Weichselbaum; the sporadic cases are frequently associated with this organism.

4. During the first week of the disease the meningococcus is present in the nasal mucus in fully half of the cases; later in the disease it is found in a smaller fraction of cases. It also occurs in the nasal secretion of some persons who are in close contact with cases of cerebro-spinal meningitis. In our series this was about 10 per cent. of the persons examined.

5. The meningococcus has a low vitality, being rapidly killed by drying and on exposure to sunlight. This makes infection by dust extremely improbable.

6. The disease seems distinctly communicable in the sense that the *organism is transmitted* from the nasal secretion of one person to another. The transmission of the organism, however, is not synonymous with *transmission of the disease*.

7. The susceptibility of the individual is an important factor in the development of the disease.

8. It seems unlikely that infection is frequently due to trauma or the result of overexertion.

9. Cerebro-spinal meningitis in other animals seems to have no connection with the disease in man. The subject, however, has not been sufficiently worked out to admit of positive statements.

10. There is no evidence to show that the disease is carried by vermin or insects.

11. The disease in some epidemics affects mostly infants, in others, older children, and sometimes chiefly adults. The reason for this is not at all clear.

12. The period of incubation seems to be short, from one to four days.

13. There is no evidence of the occurrence of "dwelling infections."

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The communicability of cerebro-spinal meningitis is also well shown by a report made to the Health Board in 1872 by Dr. Moreau Morris, City Sanitary Inspector. His report is so interesting that part of it is here reproduced.

EPIDEMIC CEREBRO-SPINAL MENINGITIS.

BY MOREAU MORRIS, M. D.,

City Sanitary Inspector.

(From the Annual Report of the Board of Health, 1871-72.)

The following report on this disease is the result of investigations and records in the Bureau of Sanitary Inspection of the Health Department, which were made by its officers during the recent epidemic which appeared for the first time, as an epidemic, in The City of New York, at the beginning of the year (1872). Isolated cases had occurred during previous years as the records of the Bureau of Vital Statistics of this Department show. Deaths had been recorded from "Cerebro-spinal Meningitis;" in 1866, 48; in 1867, 32; in 1868, 34; in 1869, 42; in 1870, 32; in 1871, 48. The statistics of the disease as recorded therein are incomplete, as some physicians failed to recognize it, from want of personal familiarity with the affection during the beginning of the epidemic; and some neglected wholly to comply with the law in respect to reporting their cases to this bureau. It may be approximately estimated that probably about one hundred cases were thus not recorded to the bureau, which eventually recovered; which estimate should enter into the percentage of deaths to all cases.

During the early part of January, 1872, reports of "new form of disease" began to reach the Bureau of Sanitary Inspection. Some called it "spotted fever," others "epidemic meningitis," a fever resembling typhoid, and typhoid complicated with acute meningitis.

* * * * *

The statistics of the disease as it prevailed in this city, and as reported to the Health Department, from January 1, 1872, to November 1, 1872, are here presented in a tabular form:

Total number of cases reported.....	990
Total number of deaths reported.....	761
Total number of houses in which cases occurred.....	835

Number of houses in which 1 case occurred.....	741
Number of houses in which 2 cases occurred.....	68
Number of houses in which 3 cases occurred.....,.....	13
Number of houses in which 4 cases occurred.....	1
Number of houses in which 5 cases occurred.....	2
Number of cases reported from hospitals included in the above total.....	60

By these statistics it appears that by far the largest proportion of cases occurred in different houses, 741 houses having but 1 case in each, out of the whole number of 835 houses in which it occurred, and this fact also strikingly illustrates the fact of its non-contagious character. Indeed it must be conceded that so large a distribution of single cases, had this disease been one of the contagions, must have added thousands to its numbers, and whole households have been stricken with it, instead of confining itself to one susceptible victim.

The greater susceptibility and fatality in youth is also strikingly exhibited in the large number attacked under 15 years of age, as shown in the accompanying table.

Total Number of Deaths from Cerebro-spinal Meningitis as Recorded in the Bureau of Vital Statistics Under Their Respective Ages and Sex, for the Period from January 1 to November 1, 1872.

	Under 1 year.	Bet. 1 and 5 years.	Bet. 5 and 10 years.	Bet. 10 and 15 years.	Bet. 15 and 20 years.	Bet. 20 and 25 years.	Bet. 25 and 30 years.	Bet. 35 and 40 years.	Over 40 years.	Total.
Males.....	60	141	81	29	23	27	13	12	18	404
Females.	56	123	56	50	18	12	13	13	16	357
Total deaths both sexes... }	116	264	137	79	41	39	26	25	34	761

* * * * *

1. Albert Brown, residing at 443 Eleventh avenue, aged 6 years and six months, was, on the forenoon of January 30, 1872, kicked in the side by a boy, and fell, striking his head against an iron railing. At 1 o'clock P. M. he reached home, but made little complaint of his injury until toward 7 o'clock that evening. He died on the morning of the 31st at 4 o'clock. No physician saw him whilst ill, and there is, therefore, no account of the symptoms.

At post-mortem examination by the Deputy Coroner, Dr. Beach, showed a thin layer of extravasated blood covering the surface of the brain, and extending to its base with bloody serum in the ventricles. Dr. B. looked on the case as one of concussion of the brain with rupture of a small vessel. There was a large patch of ecchymosis at the sight of the kick, but no signs of peritonitis or other abdominal mischief. Some dark purple spots irregularly scattered over the trunk were noticed.

2. Maximillian Brown, age four years, in good health during the day and playing up to 4 o'clock, was, at 11 o'clock P. M. February 4, 1872, seized with vomiting and spasms without loss of consciousness. He seemed in a fright and called constantly after his lost brother, case 1. He was seen at midnight by a physician. He died February 5 about 7 A. M. The medical attendant states that the brain symptoms were prominent; he did not look for any eruption. Meningitis was reported as the direct cause of death. No autopsy.

3. Theresa Brown, age 13 years, was taken, February 6, at 2 o'clock A. M. with pain in the head, moaning and crying out. She was seen by Dr. Sewell at 9 A. M. She had been sitting up during the night at her brother's "wake," and had been much affected by the sudden death of her two brothers. When first seen intelligence was perfect, the pulse rapid, the skin of natural warmth and moisture. There had been some vomiting. She complained only of pain, not severe, over the whole head. Bromide of potash was ordered, with sinapisms to the feet and nape of the neck.

February 7, 10 o'clock A. M.—She was in much distress, complaining of her head, and her mind was wandering. The pupils were somewhat dilated. Intelligence good. There was much hyperaesthesia of the entire surface, with tenderness of the large joints, which she said was rheumatism, having once suffered from it. Pulse 120 in the minute, and of good volume. The tongue was covered with thick white fur, but was not dry. She had not slept during the night; had vomited, and the bowels had been moved. At 4 P. M., she still complained of her head and limbs. The inhalation of chloroform had procured some sleep. A petechial eruption, not abundant, over the trunk and thighs. It varied in size from a pin's head to a canary seed;

did not disappear on pressure, and was of a deep purple hue. A diagnosis of "spotted fever" was made, morphia, half a grain every second hour, beef tea and milk punch were ordered. February 3, 9 A. M.—Pulse 120 and feeble; extremities cool; tongue and purpuric spots as before. Still complained of head and of great sensitiveness of the skin. Much delirium and crying out, but the intelligence was good and attention easily secured. Body near 98 Fahr., treatment continued. At 4 P. M. she was more composed; the purpuric spots were paler; face a little flushed; skin natural; pupils contracted readily to light; pulse 116 and with more volume; mind clear; tongue unchanged. February 9, 10 A. M.—Passed an indifferent night, having been restless and delirious. Pulse 130 and feeble; skin warm; tongue whitish. For the first time since her illness says that she had no pain in the head or elsewhere. Petechiae present, urine free, bowels open. Treatment continued.

At 7 P. M. the pulse was 116 and fuller; no pain; had rather an uneasy day, though she slept at intervals. Takes milk and beef tea moderately.

February 10.—Had been restless and delirious all night, and again complains of pain in head. Pulse 108 and of good character. Eruption less and of a paler hue; tongue more coated but not dry.

February 11, 2 P. M.—Had slept soundly all night; pulse 86-88; eruption nearly gone; no pain in head or back, though a little in limbs. Skin rather cool, particularly of the extremities; tongue clearer. Sitting up; taken milk and broth freely.

February 12.—Slept well; very irritable; pulse 96; a little pain in head and back of neck; eruption scarcely visible; tongue clearing; lips parched and dry; skin rather warm; urine free; clear with no change on the application of heat; bowels costive; drinks milk punch. Subsequently recovers.

4. Bertjold Brown, age 11 years. Was first seen on the morning of February 7, about 10 o'clock, lying on a couch, moribund, with a cadaveric expression, and deep icteric hue. Pulse very rapid and scarcely perceptible, skin dry and hot; mind not clear and he could hardly be roused; pupils contracted; complained chiefly of his head. He had been to his brother's funeral the previous afternoon, a distance of six or seven miles. He seemed well on his return home and ate a hearty

supper. Between 7 and 8 on the evening of the 6th of February he sickened, with pain in the head, vomiting, purging and chill. There had been no convulsions. He died at 2 P. M. February 7, eighteen hours after he was first attacked.

“Autopsy—February 8, 11 o'clock A. M.—Rigor mortis strongly marked. An eruption similar to that of the girl's (case 3) but in greater. Only on the body. The serous and mucous coat of the stomach showed purpuric spots similar to those on the body. It was also scattered, though less abundantly, over the peritoneal coat of both small and large intestines, lungs, heart, liver and kidneys were healthy. The blood was fluid. The whole of the surface of the brain was intensely congested, the veins and sinuses being gorged with very fluid blood, though not entirely devoid of coagula. On section of the brain little points of blood netted out everywhere. The ventricles were nearly dry. Consistence of brain natural. No exudation or purulent matter found.

5. February 10.—About midnight the baby, one year and three months old, was taken suddenly ill; she vomited and had several loose stools. There were slight muscular spasms, but no decided convulsions; she died at 9 A. M. The body was covered with an abundant purpuric petechial eruption.

Description of premises—This family lived on the ground floor of a house which was one of a row of wooden buildings, whose cellars had been made by filling up and grading of the avenue in front, and of the yards in rear. A good stone wall foundation had been built underneath. The apartments occupied were (a) a medium-sized front room, used as a tin shop and store; (b) a rear room, used for the general purposes of the whole family; (c) a small passageway leading from the front to the rear rooms in which some of the children usually slept in a small crib, and (d) a bed room between the rear room and shop. In this bed room which had no other means of ventilation than the door, the father, mother and some of the children slept. The whole family was thus chiefly confined to the rear room and the small unventilated bed rooms one floor on a level with the street and yard.

At No. 6 Lewis street there were 4 deaths, two girls, aged respectively 18 and 20, and two children, they all slept in a room 10 by 15 feet.

THE FREQUENT OCCURRENCE OF MENINGOCOCCI IN THE NASAL CAVITIES OF MENINGITIS PATIENTS AND OF THOSE IN DIRECT CONTACT WITH THEM.*

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Every one familiar with the investigations concerning the etiology of meningitis knows that, owing partly to the difficulty of isolating and keeping alive the meningococcus, partly to its similarity to other micrococci, the work of most investigators has been incomplete and therefore of little permanent value.

As the amount of influence which the results of the investigations here recorded may exert depends largely on the degree to which others are convinced of the thoroughness of the identification of the organisms found in the nasal cavities, it seems best to review briefly the literature in order to see what characteristics the best observers consider as belonging to the meningococcus, and therefore as necessary to prove the identity of the suspected organism.

The first important study of the etiology of primary cerebro-spinal meningitis was undertaken by Weichselbaum in 1887. Before that time it had been pretty well established that in secondary cases the pneumococcus was at times the exciting factor, though Leyden¹ and Leichtenstein² had noted diplococci in the exudate of fatal cases of primary cerebro-spinal meningitis, which they believed to be different from pneumococci. Their descriptions lead one to think that they really saw the meningococcus, but that their work was too meager to establish this..

In 1887 Weichselbaum³ isolated, and carefully studied, cultures from six typical cases of cerebro-spinal meningitis. The cocci had the following cultural characteristics: They grew well on nutrient agar-agar containing 2 per cent. of gelatin. The growth on the surface was rather flat and viscid; it was gray in direct and grayish white in transmitted light. The borders were indented and showed the growth to be made up of confluent colonies. Potato showed no visible growth. On the agar-gelatin plates the deep colonies were very small. The surface colonies were grayish white. Under the microscope they were round or irregular, finely granular, and their borders indented. They had a golden brown nucleus, an inner light yellow zone, and an outer one which was trans-

* Technical portion of Part I. of an investigation of cerebro-spinal meningitis carried on under the auspices of the Special Commission of the Department of Health of New York City.

parent and colorless. Weichselbaum found it necessary to transplant the cultures every two days in order to keep them alive, as they were found to die usually in from three to six days. The cocci themselves were mostly in pairs; some were single, and a few in tetrads and small heaps. The single cocci were round, the pairs flattened at the apposed ends. The cocci varied greatly in size and staining, the larger forms, which stained more deeply, being sometimes twice as large as the smaller, more faintly staining ones. They were mostly intracellular in the exudate, and were found only in small numbers in the tissues. All the cultures were Gram negative, and grew well only at blood heat. They did not grow at all at 20° C.

In 1895, H. Jaeger⁴ published the results of the study of fourteen cultures isolated from typical epidemic cerebro-spinal meningitis. The organisms he isolated differed from those of Weichselbaum and more recent investigators in the following characteristics: There were short chains of four to six elements present in all the cultures, and in two cultures there were long chains of twenty to thirty. He describes the cultures as being sometimes Gram positive and sometimes negative. However, he never found Gram positive cocci in the tissues. His cultures grew at lower temperature. The viability of his cultures was much greater, one culture in broth living forty-three days. The culture stood drying for ninety-six days and pus dried on linen gave a growth of the cocci after 127 days. A capsule was present in the smears.

A. Heubner,⁵ Jaeger's strongest supporter, describes cultures from four cases which were identical with Jaeger's. Jaeger, in his article of 1899,⁶ still clings to his description of 1895. In 1901⁷ he decides that the meningococcus has no capsule, but in other points holds to his original position.

Between Jaeger's first and last papers a series of investigations had been carried on which demonstrated to most bacteriologists that either he had failed to isolate the true organisms exciting the disease, or had allowed contaminating or associated bacteria to overgrow and displace the meningococci in his cultures.

Councilman, Mallory, and Wright,⁸ after a thorough study of thirty-one cases, describe their cultures as being similar to those of Weichselbaum.

In 1901, Albrecht and Ghon,⁹ after working with twenty-two cultures, agreed with Weichselbaum. The greater number of cultures observed led them to give wider limits of temperature as suitable for development. Some cultures grew from 25° to 42°, though the maximum growth was always between 36° and 37°. They are the first to describe the "bread crumb" granules found in the centre of the colony after forty-eight hours. They give the best media as Loeffler's blood serum, or agar containing ascitic fluid. A pellicle on the broth cultures, when the broth was neutral and the cultures were left quiet for several days, was almost constant. In a few instances they kept cultures alive, when protected from drying, for 185 days without transplanting. All the cultures were Gram negative, and there was no tendency to chain formation. Albrecht and

Ghon obtained cultures from Jaeger and from Heubner, and found them not only quite different from theirs, but also unlike each other.

Albrecht and Ghon,¹⁰ and Weichselbaum,¹¹ in convincing articles published in 1903, take up the peculiarity of Jaeger's cultures point by point, and are of the opinion that he was not working with true meningococcus cultures. Taking the important points agreed upon by the best workers, Albrecht and Ghon give the following characteristics as essential in identifying true meningococcus cultures:

1. Gonococcus-like in form, dividing in the same way, always Gram negative, having many degeneration forms, and often intracellular.
2. Growing only at fairly high temperature, 25° to 42°, maximum growth at 36° to 37°.
3. Colonies on agar plates luxuriant, quite viscid, glistening, gray in direct light and grayish white in transmitted light.
4. Growth confined almost entirely to surface in stab culture.
5. Develops pellicle on broth culture (when the broth is neutral and the cultures are undisturbed for several days).
6. Slight pathogenicity for ordinary animals.
7. Non-resistant.

MICROCOCCUS CATARRHALIS.

In 1901, Ghon and H. Pfeiffer¹² published the results of the study of forty cultures of *Micrococcus catarrhalis*. They found that, while it grew best on blood agar, it would grow on ordinary media. It differed from the meningococcus in growing more easily, more luxuriantly, and at a lower temperature. The colonies under the microscope were darker, more compact, and had more abrupt margins. Jaeger¹³ finds all the strains of *M. catarrhalis* self-agglutinating. Some of the cultures examined by us have had all the above characteristics, while others have more nearly resembled the meningococcus.

MENINGOCOCCUS CULTURES ISOLATED BY PREVIOUS INVESTIGATORS FROM THE NASAL MUCUS.

In going over the literature we are impressed with the small number of cases from which thoroughly identified meningococcus cultures have been isolated from the nasal mucus. The cases from which Gram negative diplococci closely resembling meningococci have been found in the smears from the nose and throat are, on the contrary, numerous and have been found by nearly all workers on meningitis.

The first to identify as meningococcus a culture taken from the nasal mucus was F. Kiefer.¹⁴ While working with meningitis cultures, he developed a severe purulent rhinitis. The pus contained numerous meningococci. In 1898, Schiff¹⁵ isolated cultures from three out of twenty-nine dispensary patients, a portion of

whom suffered from chronic laryngitis, which cultures, he says, Weichselbaum considered true meningococci. These three cultures will be considered later in connection with two obtained by us from medical students, which agreed with the meningococci obtained from the spinal fluid in all respects except in agglutination characteristics. Councilman, Mallory, and Wright⁸ report one culture from the throat of a tonsillitis case. Griffon and Gandy¹¹ twice, at an interval of five days, isolated cultures from the nose of a meningitis case which were identical with cultures from the spinal fluid. Albrecht and Ghon⁹ report two instances, one a case of meningitis, the other from a man whose child died of meningitis three days before the culture was taken. F. Lord,¹² of Boston, isolated meningococci from a case of rhinitis. A. Weichselbaum and Ghon¹⁰ identified one culture from the nose of a meningitis patient and three from the noses of people in contact with patients. These cultures from the fourteen cases were the only ones we could find that were studied with sufficient care to warrant their acceptance as true meningococci.

ORIGINAL INVESTIGATION.

Most of the material for this investigation was obtained through the courtesy of Dr. A. W. Taves, of Gouverneur Hospital.

The mucus was taken from the nasal fossæ with a sterile cotton swab and plated out as soon as possible on ascitic agar. As a rule the plates were made within one hour of collecting the specimen, while the swab was still moist. These plates were incubated from 24-48 hours, then fished in the usual way. The colonies were put on blood agar, which seemed to be the most favorable medium.

Several colonies were fished from every type found which resembled a meningococcus colony in color or granularity, and which under the high power showed diplococci resembling meningococci. The organisms from the cultures were stained by Gram, and several of the Gram negative ones, which in cultures resembled meningococci were kept for study.

Fifty-two meningitis cases were examined. Meningococci were isolated from 12 of the 22 cases examined during the first week, and from five of the 15 examined during the second week. In six cases examined during the third week, three during the fourth, and six between the fifth and ninth weeks, no meningococci were found, while in a very severe case examined on the 67th day we found a few

colonies. In one case we failed to get them on the first day and found them in large numbers on the second.

From this it would seem as though the meningococci were present in a rather large percentage of the cases during the first week.

The nasal secretions of 45 healthy persons living in close contact with meningitis patients were examined. In five of these meningococci were isolated during the first two weeks of the patients' illness. From the nasal mucus of 55 medical students who had never been in known contact with meningitis there were isolated in two cases a few organisms which were, culturally and in inoculation experiments, like meningococci.

In studying the agglutination of these cultures from medical students, we found that they differed from our other cultures in their specific agglutinins, and therefore were differentiated in one important respect from them. In this connection it is of interest that Schiff, in describing his cultures from the nasal cavity of people not in contact with meningitis, does not refer to agglutination, and evidently did not make the test. His cultures may have differed as ours do. One cannot safely classify these atypical cultures. They may be meningococci derived from strain different from those isolated by us in the present epidemic, or organisms not capable of readily exciting meningitis, and yet so closely related that they cannot be differentiated without more careful cultural tests than we at present use.

The following tables give the cases, the day of the disease when the specimen was taken, the termination of the disease and the bacteriological findings.

TABLE I.

Cases of Meningitis in Which Meningococci Were Isolated from the Nasal Mucus.

Name	Day of Disease.	Termination.	Percentage of Meningococcus Colonies Present in Plates.
W. W.....	1	Died	About 55
J. N.....	2	Died 3d day	" 90
L. Z.....	3	" 30
E. R.....	3	Died 4th day	" 50
R. T.....	3	Died	" 40
J. G.....	4	" 95
Mrs. M.....	4	Died 6th day	Very few
S. F.....	5	Died	About 50
S. K.....	5	" 90
J. S.....	5	" 30
D. M.....	7	Recovered	" 2
C. P.....	7	Few
M.....	10	Died	About 10
M. G.....	10	Died	" 2
J. M.....	12	Died	A very few
M. H.....	14	About 95
E. S.....	14	Died	" 5
S. K.....	67	Died 69th day	" 2

Contacts with Meningitis Cases from Whom Meningococci Were Isolated from the Nasal Mucus.

Name.	Day of Contact.	Condition.	Percentage of Meningococcus Colonies Present in Plates.
Mr. D.....	4	Normal	About 95
Mrs. D.....	4	"	" 95
Mrs. K.....	14 still in contact	"	" 95
A. K.....	14 "	"	" 50
Mrs. M.....	14	"	" 30

The plate cultures from the mucus of all these cases contained many colonies and in most cases great numbers of colonies.

TABLE II.

Cases of Meningitis in Which Meningococci Were Not Isolated from the Nasal Mucus.

Number of Cases.	Day of Disease.	Number of Cases.	Day of Disease.	Number of Cases.	Day of Disease.
1	1	2	13	1	24
1	2	5	14	1	27
2	3	1	15	1	28
5	6	1	17	1	31
1	7	1	18	1	40
1	9	1	19	1	42
1	10	1	20	1	49
1	11	1	21	1	60

TABLE III.

Contacts with Meningitis Cases from Whom No Meningococci Were Isolated from the Nasal Mucus.

Number of Persons.	Days Since Contact.	Number of Persons.	Days Since Contact.
5	2	1	35
9	3	1	50
2	4	3	56
1	10	1	60
1	18	16	Still in contact.

All contacts were occupants of the same rooms, and nearly always members of the family.

From 14 cases we took multiple specimens. In only one case did we find meningococci in two specimens, 90 per cent. on the fifth day, and a very few on the tenth. The following table gives the cases, the day of disease and the bacteriological findings:

TABLE IV.
Cases of Meningitis from Which Multiple Specimens Were Examined.

Name.	Day of Disease.	Termination.	Findings.
B. I.....	13	Recovery.	No Meningococci.
B. I.....	14	"
B. I.....	15	"
B. I.....	16	"
E. E.....	2	Died 7th day.	"
G. D.....	3	"
G. D.....	7	Died 45th day.	"
G. D.....	13	"
G. D.....	19	"
K. S.....	5	90 per cent.
K. S.....	10	A few.
K. S.....	16	No Meningococci.
M. D.....	7	Recovered.	2 per cent.
M. D.....	13	No Meningococci.
M. D.....	19	"
C. P.....	3	"
C. P.....	4	5 per cent.
C. P.....	5	No Meningococci.
C. P.....	7	"
C. P.....	8	"
C. P.....	9	"
S. M.....	2	"
Si M.....	6	"
S. M.....	8	"
S. J.....	6	Recovered.	"
S. J.....	7	"
S. J.....	9	"
S. J.....	10	"
S. T.....	3	Died 10th day.	"
S. T....	4	"

Name.	Day of Disease.	Termination.	Findings.
S.J.	5	30 per cent.
S J.....	10	No Meningococci.
S. J.....	18	"
W. F.....	3	"
W. F.....	4	"
W. J.....	17	"
W. J.....	18	"
W. J.....	19	"
W. J.....	20	"
W. J.....	22	"
Z. L.....	1	"
Z. L.....	3	30 per cent.

CULTURAL CHARACTERISTICS OF THE MENINGOCOCCI ISOLATED FROM THE NASAL MUCUS.

The cultures isolated from the nasal mucus were carried out on the different laboratory media and compared with 30 cultures isolated from a similar number of specimens of spinal fluid.

There were no apparent differences between the nose and spinal fluid cultures. Some grew more luxuriantly than others. The more luxuriant cultures from both spinal fluid and nose seemed to have a more yellow tone, while those growing in a thinner layer were grayish white.

The morphology of the organisms differed slightly, but the differences were the same for cultures from both sources.

The meningococci occurred as flattened cocci in pairs, fours, and sixes. They varied widely in size in the same culture from the same media, and differed greatly in the intensity with which they took the stain.

In no case did a culture tend to be Gram positive. Cultures were repeatedly plated out, and numerous colonies fished and stained by Gram. In a culture transplanted twice a day for five days on Loeffler's blood serum, so that the organisms might all be very young; there was no tendency for any of them to be Gram positive.

In no culture was any tendency to chain formation observed. The cultural characteristics of colonies on ascitic agar plates were as follows:

1. *Macroscopic appearance*.—In many cultures there are two distinct zones, but this was not found constant on repeated plating. Where the colonies are in contact, they are usually divided by a distinct line. They are oval or irregular, grayish-white to yellowish-white, moist and unusually viscid, flowing about the needle instead of breaking away from it when they are fished.

2. *Microscopic appearance: Low power*.—Pale amber to brown in color. From fine and evenly granular colonies to those with very coarse central granules. Margins generally rather even and often not abrupt.

3. *Microscopic appearance: High power*.—The diplococci and occasionally the fours show plainly. On some plates the margins are smoother and abrupt, and the separate organisms are distinguished with difficulty.

The most constant characteristics seem to be the coarse central granules and the characteristic separate organisms at the margins when observed with higher power.

Ascitic agar slants.—Grayish white, fairly luxuriant growth, usually with discrete colonies. The colonies at times have a diameter of five millimeters at forty-eight hours. They are generally quite round, but vary a good deal in the waviness of their outlines. Two zones are often distinguished. In the smears from ascitic agar the organisms stain poorly and are indistinct.

Loeffler's blood serum.—The growth is heavy, moist, confluent and yellowish. The smears show the organisms distinctly, and usually of larger size than on ascitic agar.

Plain agar.—Growth scant, if any, and generally consisting of a few isolated colonies.

Glucose agar.—Growth slightly better than on plain agar.

Glycerin agar.—Same as plain agar.

Blood agar.—Growth very luxuriant, confluent, yellowish white and extremely sticky; smears same as from Loeffler's.

Sheep serum agar.—Growth fairly luxuriant, about the same as ascitic agar.

Gelatin.—No cultures grew below 24°. At 37° C. all the cultures grew well, with the formation of a heavy pellicle. At the end of six weeks the gelatin still hardened when put in the ice box.

Hiss's inulin medium.—Rendered opaque but not coagulated.

Litmus milk.—The cultures grow only slightly and turn the milk somewhat darker than control at the end of forty-eight hours, but make no further change.

Marble broth.—Most of the cultures grow slightly, a few grow well, making the medium cloudy, afterward forming a pellicle and sediment. The pellicle is quite general after one week.

Plain broth.—Very few cultures grow in our broth and these only slightly. This was possibly due to an unsuitable reaction of the broth.

Dunham's peptone solution.—Growth very slight. Indol not produced.

Glucose litmus peptone sheep serum agar.—Acid produced after forty-eight hours.

Lactose litmus peptone sheep serum agar.—Acid not produced after forty-eight hours.

Maltose litmus peptone sheep serum agar.—Acid produced after forty-eight hours.

Saccharose litmus peptone sheep serum agar.—Acid not produced after forty-eight hours.

Mannite litmus peptone sheep serum agar.—Acid not produced after forty-eight hours.

Temperature.—The maximum growth was about 37°. Nearly all the cultures grew at 30° three months after isolation; a few grew slightly at 24°.

Viability.—The cultures varied greatly in the length of time which they would live without transplanting. In order not to lose cultures, we reinoculated them every five days. Many of the cultures on ascitic agar lived from ten to twenty days without protection from drying, and some of the broth and gelatin cultures lived from five to eight weeks. After twenty-five cultures were kept in the ice box for five days, none of them were alive. Cultures left at room temperature and in the ordinary amount of light varied greatly in their resistance. Most of them failed to grow after forty-eight hours.

AGGLUTINATION.

Albrecht and Ghon⁹ and Bettencourt and Franca²⁰ found that the serum of meningitis patients agglutinated meningococci in from 1.10 to 1.100 dilutions. They found that the serum of animals immunized for a long time with meningococci agglutinated the cultures only in low dilutions, 1.100 being the highest. We tested the serum of very few patients. The highest dilution agglutinating was 1.200.

Finding it impossible to distinguish between nasal and cord cultures by morphological or cultural comparison, we have made use of a specific serum to aid in classifying the cultures from the different sources.

We inoculated two horses, two sheep, three goats and 20 rabbits. Only two rabbits lived long enough to give a serum of sufficient agglutinating strength to help in our work. Of these two, one was inoculated with W., a nasal culture from a student not in contact with meningitis. This serum agglutinated its own culture and several typical meningococcus cultures completely in a dilution of 1.40. The other was inocu-

lated with a cord culture, and agglutinated its own culture in a 1.400 dilution, and other cultures in a 1.50 or slightly higher dilutions.

One sheep, after being inoculated with rather large doses of a cord culture for over three months, gave a serum agglutinating most of the cultures completely in a 1.40 dilution. The goat sera never agglutinated above 1.20.

One horse was inoculated with a nasal culture obtained from a severe case of meningitis on the second day of the disease. The patient died on the third day. This horse died after a month's treatment, before the serum was of much value. The other horse was given a cord culture, and though he became very sick at the end of the first month he improved when given smaller doses. At the end of four months the agglutinating strength of this serum was 1.100 for most of our cultures. It seemed better for some other cultures than for its own.

There was a great difference in the degree of agglutinability of the cultures on different days, which made it very difficult to compare the results quantitatively.

The following tables give some of the serum tests with cultures from the spinal fluid and noses of patients, and from the noses of contacts and from people not in contact. As a rule, the majority of the cultures seem to agglutinate as well as the culture with which the animal was inoculated.

TABLE V.

Agglutination of 22 Cultures Obtained from the Spinal Fluid, and of 21 from the Nasal Mucus by Sheep 182 Serum After Animal Had Been Inoculated for Three Months.

	Control.	1:20	1:50	1:100	1:200	1:400
33-2n	—	+	++	+	+	—
XI-2n	—	+	++	+	—	—
124-1c	—	+	+	±	1	—
VII.-3n	—	+	+	1	—	—
W. P. 1c	—	+	+	±	—	—
108-5c	—	+	+	±	—	—
D. Getz c	—	+	+	±	1	—
Wiesbard c	—	+	+	+	—	—
100-2c	—	+	+	±	1	—

	Control.	1:20	1:50	1:100	1:200	1:400
95-2n	—	±	I	—	—	—
140-2c	—	+	I	±	—	—
91-1n	—	+	+	+	I	—
114-2n	—	+I	±	—	—	—
152-1c	—	+	+I	±	I	—
M142-2n	—	+	±	±	±	—
Preglia c, 140-3c	—	+	+	±	I	—
Stolz-2n	—	+	±	I	—	—
Cohen c	—	±	±	±	I	—
Fieland cord c	—	+	+	+	I	—
Fieland nose n	—	+	+	+	±	—
Goldfarb cord c	—	+	+I	+	—	—
Schwartz n	—	+	+	—	—	—
Goldfarb nose n	—	+	±	±	—	—
182 s. c	—	++	++	—	—	—
Merrit n	—	+	±	I	—	—
136 s. c	—	+	+	+	I	I
23-2n	—	++	+	—	—	—
1X.-2n	—	+	—	—	—	—
Horrowitz c	—	—	—	—	—	—
14c	—	++	—	—	—	—
105-5n	—	+	+	±	—	—
253-5c	—	+	—	—	—	—
36-8n	—	++	++	—	—	—
29-3n	—	+	+	+	±	I
85-1n	—	+	+	I	—	—
142-s. c	—	+	±	—	—	—
Bayridge c	—	+	—	—	—	—
Rubin n	—	±	+	±	I	—
Mersu n	—	+	+I	±	I	—
Fielder c	—	+	+	+	—	—
Gruno c	—	++	++	+	I	—
McDonald n	—	±	+	I	—	—

In testing the agglutinating power we used emulsions made from 24-hour sheep serum agar slants in normal salt solution. We used hanging drops, with the slides inverted until the moment of examination, to prevent mistaking mechanical grouping for agglutination. The hanging drops were usually examined after four hours and marked in the following way: — = no agglutination, I = trace, ± = marked trace, + = good agglutination, +I = very good agglutination, ++ = complete agglutination.

c. Indicates culture isolated from spinal fluid.

n. Indicates culture isolated from nasal mucus.

We saturated the best horse serum with its own culture, with nasal cultures from a severe meningitis case, with a contact with a non-contact, and with several *M. catarrhalis* cultures. After allowing the mixture of serum and culture in a 1:5 dilution to stand for three hours, we filtered through a Berkefeld filter, and used the third 10 c.c. of the filtrate. All the meningococcus-like cultures seemed to remove the agglutinins for all the cultures, while the *M. catarrhalis* cultures only reduced them about one-third. The control filtration of the serum without exhaustion reduced the agglutinins about as much as the *M. catarrhalis* cultures.

TABLE VI.

Tests of the Serum of Horse 277, After Being Inoculated for Four Months with 142 S., a Spinal Fluid Culture.

	Serum.		Serum Extracted with XI-2, a Meningococcus Culture from the Nasal Mucus of a Meningitis Patient.			Serum Extracted with a <i>M. catarrhalis</i> Culture from a Meningitis Case.		
	1:20	1:40	1:5	1:10	1:20	1:5	1:10	1:20
Gruno c.....	+	+	—	—	—	+1	+	1
Fielder c.....	+	+	—	—	—	+1	+	1
142 Sc.....	+	+	—	—	—	++	+	+
XI-2 n.....	+	+	—	—	—	++	1	1
33-2 n.....	+	+1	—	—	—	++	1	1
36-8 n.....	±	±	—	—	—	++	—	—
W. n.....	+	+	+1	1	1	+	+	±

These sera, after being extracted, were in a 1:5 dilution filtered through a Berkefeld filter and the third 10 c. c. used.

We saturated this same horse serum with a meningococcus culture, with a *M. catarrhalis* culture, and with W. n. from a student, a non-contact case. Instead of filtering we centrifuged, and found our results somewhat different. The meningococcus culture took out all the agglutinins for the meningitis culture and not for the others, while the *M. catarrhalis* and the W. n. left in over half the agglutinins for the meningitis cultures. The *M. catarrhalis* agglutinated spontaneously, but the non-contact W. n. took out all of its own agglutinins.

TABLE VII.

Tests of the Serum of Horse 277, After Being Inoculated for Four Months with 142 S., a Spinal Fluid Culture.

	Serum Unextracted.		Serum Extracted with a Spinal Fluid Culture.		Serum Extracted with W. n., a Culture from a Person Not in Contact with Meningitis.		
	1 : 100	1 : 200	1 : 10	1 : 20	1 : 20	1 : 40	1 : 100
Gruno c	+	—	—	—	+	1	—
Fielder c	+	—	—	—	+	1	—
142 Sc	+	—	—	—	+	1	—
XI-2 n	+	—	—	—	+	1	—
33-2 n	+	—	—	—	+	1	—
36-8 n	+	—	—	—	+	1	—
W. n	+	—	—	—	—	—	—

The sera, after being extracted, were centrifuged instead of filtered.

PATHOGENICITY.

Weichselbaum, in 1887, with his original cultures, killed white mice with an intraperitoneal or intrathoracic inoculation of 5 c.c. of a broth dilution of an agar culture or the water of condensation. The mice died in 36-48 hours, and the meningococci were found in the cavity inoculated and usually in the blood. Subcutaneous inoculations were without result. He killed guinea-pigs by inoculating them in the thoracic cavity; but the cocci were not found in the blood or spleen.

Three dogs inoculated subdurally with 1 c.c. and 1.5 c.c. of culture dilution died, one the same evening, the second on the third day, and the third on the 12th day. The first two showed a small amount of fluid blood between the dura and brain. There was a small area of punctiform hemorrhages deeper in the brain, and the membranes were markedly injected. Numerous meningococci were found. In the third day, between the dura and the right cerebral hemisphere, there was thick red pus, and in the brain a hazel-nut sized abscess containing yellow pus. Around the abscess was a hemorrhagic area. The lateral ventricles contained a red fluid with flakes of pus. No meningococci were found.

Albrecht and Ghon inoculated a goat in the spinal canal, which developed symptoms of meningitis and died in five days. The cord showed no changes and meningococci were not isolated.

Our animal work was rather irregular in its results. By inoculating mice intraperitoneally with .5 of a 24-hour ascitic agar culture of either the cord or nose strains, we caused death in 24-48 hours. There was marked congestion of the abdominal viscera, and meningococci were found in the blood and peritoneal exudate.

Rabbits were very uncertain. A few died from subdural inoculation of rather large doses, but there were no typical lesions, and none of them contained meningococci in the blood or exudate.

With small puppies we obtained about the same results as Weichselbaum. When given a dose of two ascitic agar cultures in the spinal canal, the dogs usually died in 24 to 48 hours. They had convulsions and some rigidity of the neck. On autopsy the membranes were much injected and there were hemorrhagic areas in the cortex. Meningococci were found in these areas, in the fluid under the dura, and in the spinal fluid.

As controls to our meningococcus cultures, we used *M. catarrhalis* cultures and two cultures corresponding culturally to meningococci, which had been isolated from the nasal mucus of normal medical students. The dogs inoculated with two ascitic agar cultures of *M. catarrhalis* did not die, while those which received the cultures from the students died in 24 hours, and gave the same autopsy results as the dogs inoculated with meningococci.

CONCLUSIONS.

Meningococci were isolated from the nasal mucus of 50 per cent. of meningitis patients during the first two weeks of the disease and from about 10 per cent. of the people most closely in contact with them. They were frequently present in enormous numbers.

The two cultures isolated from normal students were like meningococci culturally and in their pathogenicity, but did not have the same specific agglutinins.

The finding of meningococci in great numbers in the nasal mucus of such a large proportion of the patients and of those caring for them,

and the absence of meningococci from the nasal mucus of a large number of normal persons examined, would strongly indicate the necessity of isolating cases of epidemic cerebro-spinal meningitis, at least during the early weeks of the disease.

We wish to thank Dr. Park for his constant oversight and direction of our work.

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THE VIABILITY OF TYPHOID BACILLI IN OYSTERS,

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During the past year a series of experiments have been conducted to determine the length of time typhoid bacilli may remain in oysters under market conditions. The oysters were placed in water, which had been previously sterilized, and to which, after sterilization, a certain number of typhoid bacilli were added. The oysters were allowed to remain in this water 24 hours, when they were removed and kept on ice as they are kept under market conditions. Where there were a large number of typhoid bacilli added to the water (50,000 or more per cc.) typhoid bacilli were easily isolated at intervals up to six weeks. At the end of this time, most of the oysters had undergone degenerative changes, and were no longer fit for assimilation. Where the water was infected with a very small number of typhoid bacilli (75 or less to a cubic centimeter) the typhoid bacilli could not be identified owing to the excessive growth of other micro organisms, principally moulds. I infected one set of oysters with *B. coli* (43 to a cubic centimeter). In this lot of oysters, I could easily determine the presence of bacillus coli, in 1/10 of a cubic centimeter of this juice. They were no longer fit for the market. It would seem, therefore, that there was a possibility of typhoid bacilli remaining in oysters when once infected, even though in small numbers.

I have noticed in a few oysters undergoing degenerative changes that the typhoid bacilli increase rather rapidly. This, however, was not constant. Some of these oysters were still in condition to be sold.

Number of Lot.	Period between Infection and Test.	Number of Organisms in the Normal Oyster Juice per c. c.	Number of Organisms in the Infected Water. (B. Typhosus.)	Number of Organisms per c. c. of Juice of Oysters after Infection.	Number of Colonies Fished.	Percentage of Colonies identified as Typhoid.	Remarks.
IIIa.....	24 hours.....	1,500-2,000	250,000,000	1,125,000,000	Plates too crowded to fish. The other oysters had died, and were too dry to test..
"	6 weeks.....	800,000,000	
"	6 "	75,000,000	60	100	
IIIb.....	24 hours.....	50,000,000	93,700,000	60	100	Others all dead, and too dry to make test.
"	6 weeks.....	23,600,000	20	100	
IIIc.....	24 hours.....	1,500-2,000	15,000,000	67,500,000	20	100	
"	2 weeks.....	80,000	20	100	
"	3 "	20,000	20+	44	
"	3 "	250	20+	30	
"	4 "	35,000	20+	70	
"	6 "	48,000	20+	42	Oysters dead; very slight change.
Lot 2.....	24 hours.....	2,500-3,000	10,000,000	8,000,000	20+	36	
"	24 "	32,000,000	37	100	
"	4 days.....	2,572,000	32	100	Oysters dead; only sign heart not breathing.
"	4 "	162,400,000	25	60	
"	2 weeks.....	4,000,000	25	100	
"	2 "	20,000,000	15	100	Oysters sound; normal.
"	3 "	5,500,000	18	100	
"	4 "	5,000	20	88	
"	5 "	70,000	20	70	No test for B. Typhosus.
I.....	24 hours.....	30,000	500,000	400,000	20	66	
I.....	5 days.....	100,000	

Number of Lot.	Period between Infection and Test.	Number of Organisms in the Normal Oyster Juice per c. c.	Number of Organisms in the Infected Water. (B. Typhosus.)	Number of Organisms per c. c. of Juice of Oysters after Infection.	Number of Colonies Fished.	Percentage of Colonies identified as Typhoid.	Remarks.
Lot 3.....	24 hours.....	1,500-2,000	64,000	112,500	20	100	No test for B. Typhosus.
"	2 weeks.....	92,000	50	58	
"	3 "	78,000	30+	47	
"	4 "	121,000	25+	75	
"	5 "	74,000	25+	61	
"	6 "	41,000	25+	23	
Lot 4.....	24 hours.....	3,000-16,000	73	2,500	50	No oysters living at the end of this period.
"	24 "	20,000	50	
"	24 "	No B. Typhosus found at any time in 24 hours.			
Lot 5.....	24 "	6,000-7,000	52	"	"	" 18	
Lot 6.....	24 "	2,000-21,000	61	"	could be isolated in 20 oysters.		
Lot 7.....	24 "	2,800	43 Colon B.	2,300	Coli in 1/10 of a c. c.		
"	5 days.....	2,800	2,900	"	"	
"	12 "	3,100	"	"	
"	2 weeks.....	3,400	"	"	
"	18 days.....	1,400	"	"	
"	21 "	1,800	"	"	
"	24 "	1,700	"	"	
"	28 "	2,400	"	"	
"	31 "	2,450	"	"	
"	38 "	2,000	"	"	

A COMPARATIVE STUDY OF ACCURATE AND ROUGHLY
ESTIMATED DILUTIONS OF DRIED BLOOD IN THE
TEST FOR SUSPECTED TYPHOID FEVER.

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During October, November and part of December, 1905, all of the dried specimens sent in for the Widal test for typhoid, which were reported positive by the diagnostician at the laboratory at Fifty-fifth street, were sent to us daily for corroboration. The object here was to make as accurate dilutions as possible in order to discover how much discrepancy existed between the results so obtained, and those obtained by the rough dilutions used in the usual routine examination. Our technique was as follows:

The dried blood was carefully scraped from the slide into a small vial of known weight, and weighed on an analytical balance. Previously by experiment, we found that about 70 per cent. in weight was lost by the fresh drop of blood in drying, irrespective, after 24 hours, of the length of time dried. To the weight of the estimated fresh drop of blood was added the necessary weight of sterile distilled water to make the dilution required. The water was measured by means of fine capillary pipettes, the drops from which were found by experiment to be of constant weight. On account of the minute quantity of the materials dealt with, we were compelled to use hanging drop rather than test tube reactions.

The organism used was the so-called Pfeiffer bacillus, which was obtained from Prof. Pfeiffer ten years ago, and is peculiarly adapted for agglutination work on account of its sensitiveness to reactions. A 24-hour broth culture diluted one-half with sterile 8/10 per cent. salt solution was used after the first week, as it was found with this we got the best results. During the first month, we did not systematically test with the paratyphoid bacillus, as we had some difficulty in getting organisms which did not agglutinate spontaneously. We finally after transferring daily for some time, got two strains of paratyphoid—Normach and Buxton, which were satisfactory. In all there were 166 specimens of blood examined, of which 131 were positive and 35 negative. Among

the negative, we include not only those where there was no agglutination, but also those which gave only a marked tendency in 1:25 dilution. The hanging drops were examined at the end of one-half hour, one hour and two hours respectively, and the reading in the table is that of the highest degree of agglutination, usually at the end of two hours. The symbols used to designate the degree of reaction are those commonly used by us in agglutination work, viz: + + complete reaction, +| very good agglutination, + good, \pm marked tendency, | slight and — no agglutination.

The reactions obtained at the diagnostic laboratory were not sent down to us until after the readings were made.

The following table which gives part of our results will serve to show the results obtained by the two methods. On comparing the results obtained by the two methods of dilution, one is struck by the surprising accuracy of the rough test. Out of the 166 specimens of blood examined, there were only seven negative results obtained by the process of accurate dilutions by weight against the positive results reported at the Fifty-fifth Street Laboratory. All of the specimens sent down as "negative," were corroborated by us.

Number.	Diagnosis.	Duration.	Reaction at 55th Street.	Typhoid (Pfeiffer)							Paratyphoid.		
				1 to 25	1 to 50	1 to 100	1 to 200	1 to 400	1 to 800		Normach.		Buxton.
											1 to 25	1 to 50	
Nov. 21, 1905													
" 1, "	10 days	+	++	+	+	-	-	..		-
" 2, "	Remittent Fever..	8 "	+	+	++	+	+	+	+		+	+	1
" 13, "	Typhoid.....	7 "	+	++	+	-	-	-	..		-
" 14, "	14 "	+	+	-	-	-		-
" 16, "	8 "	+	1	-	-	-		-
Nov. 23, 1905													
" 4, "	Typhoid.....	2 weeks	+	++	+	+	-	-	..		-
Nov. 24, 1905													
" 8, "	Typhoid.....	14 days	+	+	+	+	+	-	-		+	+	+
" 10, "	Typhoid.....	10 "	+	+	+	+	-		-	-	-
" 13, "	21 "	+	+	1	-	-		+	-	-
Nov. 27, 1905													
" 10, "	Paratyphoid.....	4 weeks	+	+	+	1	-	-	..		-	-	- 1-100
" 12, " (16 Nov. 21)...	Typhoid.....	3 "	doubt-ful	1	-	-	-		-	-	..
" 14, "	Typhoid.....	12 days	+	++	+	+	-	-	..		1	-	-
" 16, "	2 weeks	+	++	+	+	1	-	..		1	-	-
Nov. 29, 1905													
" 17, "	{ Malaria, No. 16 } Nov. 20.....	-	-	-	-		-

Number.	Diagnosis.	Duration.	Reaction at 55th Street.	Typhoid (Pfeiffer).								Paratyphoid.		
												Nornach.		Buxton.
				1 to 25	1 to 50	1 to 100	1 to 200	1 to 400	1 to 800	1 to 25	1 to 50	1 to 25	1 to 50	
Dec. 1, 1905														
" 8, "	12 days	+	..	+	+	1	-	-	-	..	-	-	..
" 21, "	Typhoid relapse..	42 "	+	..	+	+	+	-	-	-	..	-	-	..
" 28, "	7 "	+	1	1	-	-	-	..	-	-	..
" 29, "	7 "	+	++	++	+	+	+	1	-	..	-	-	..
Dec. 5, 1905														
" 7, "	2 weeks	+	++	++	+	+	1	-	-	-	+	+	1
" 20, "	Typhoid.....	10 days	+	+	1	-	-	-	..	-	-	..
Dec. 6, 1905														
" 8, "	9 days	+	++	++	+	+	+	1	-	-	+	+	1
" 14, "	5 "	+	++	+	-	-	-	-	1	-	-
" 16, "	Typhoid.....	8 "	+	++	++	+	1	-	..	-	-	+	+	1
Dec. 11, 1905														
.....	Typhoid.....	3 weeks	+	+	+	+	1	-	-	-	-	+	+	..
.....	Typhoid.....	9 days	+	++	++	+	+	1	1	-	-	+	+	..
.....	11 "	+	++	+	+	+	1	-	-	-	-	-	..

REPORT OF BACTERIOLOGICAL EXAMINATION OF WATER SPECIMENS FOR THE YEAR 1905..

BY MARY E. GOODWIN M. D.,

Bacteriologist, Research Laboratory.

Croton tap water at East Sixteenth street was plated in agar and tested for the presence of colon bacilli once a week during the year. The colony count at 37 degrees and 24 degrees was as follows:

	1 c. c. Plated in Agar at 37° for 24 Hrs.	At 24° for 72 Hrs.	Quantity of Water Containing Colon Bacilli as Shown by the Presumptive Test.
January 3.....	50 colonies	1,624 colonies	1 c. c.
" 10.....	70 "	3,760 "	1 "
" 17.....	60 "	1,992 "	10 "
" 24.....	76 "
February 16.....	14 "	324 colonies	10 c. c.
" 22.....	25 "	1,600 "	1 "
" 28.....	18 "	928 "	10 "
March 7.....	19 "	528 "	10 "
" 14.....	148 "	7,000 "	10 "
" 22.....	85 "	1,500 "	10 "
" 28.....	82 "	792 "	1 "
April 6.....	40 "	480 "	10 "
" 12.....	856 "	10 "
" 19.....	23 colonies	200 "	10 "
" 26.....	30 "	9,000 "	10 "
May 2.....	29 "	489 "	10 "
" 9.....	108 "	424 "	10 "
" 16.....	56 "	175 "	10 "
" 23.....	162 "	460 "	10 "
" 30.....	15 "	235 "	1 "
June 6.....	28 "	600 "	10 "
" 13.....	36 "	150 "	0.1 "
" 20.....	60 "	278 "	1 "
" 29.....	108 "	416 "	0.1 "
July 5.....	425 "	624 "	1 "
" 11.....	154 "	330 "	1 "
" 19.....	16 "	100 "	1 "

	1 c. c. Plated in Agar at 37° for 24 Hrs.	At 24° for 72 Hrs.	Quantity of Water Containing Colon Bacilli as Shown by the Presumptive Test.
July 25.....	3,040 colonies.....	9,100 colonies.	0.1 c. c.
August 1.....	125 "	140 "	1 "
" 8.....	56 "	112 "	0.1 "
" 15.....	143 "	199 "	0.1 "
" 22....	125 "	135 "	0.1 "
" 29.....	200 "	236 "	0.1 "
September 6.....	88 "	160 "	*0.01 "
" 14.....	420 "	890 "	*0.01 "
" 20.....	320 "	150 "	0.1 "
" 27.....	108 "	300 "	0.1 "
October 3.....	190 "	630 "	*0.01 "
November 14.....	170 "	550 "	1 "
" 22.....	150 "	1,300 "	0.1 "
" 28.....	350 "	2,530 "	0.1 "
December 5.....	160 "	9,000 "	10 "
" 12.....	470 "	6,480 "	1 "
" 27.....	470 "	20,000 "	1 "

* Specimens taken from tank on roof.

During the year three trips were made to the Croton water shed to investigate the possible sewage contamination of the streams entering into Croton lake, and the condition of Croton lake.

At all times the sewage of Mt. Kisco was found to enter almost directly into Branch Brook, which enters into Croton lake by way of Kisco river. The water at the surface of the lake contained fewer bacteria to the c.c. than did the water at the outlet where more of the sediment was present.

On June 24, the following specimens were taken from the Croton water shed:

- No. 1—The filtrate from Mt. Kisco sewer beds.
- No. 2—Hotel and storm drain.
- No. 3—Branch brook below drain.
- No. 4—Kisco river.
- No. 5—Brook from Italian settlement.
- No. 6—Croton dam surface water.
- No. 7—Croton lake outlet.

1 c.c. Plated in Agar.	At 37° 24 Hours.	At 24° 72 Hours.
No. 1.....	125,000 colonies	350,000 colonies.
No. 2.....	Plates uncountable.....	Plates uncountable.
No. 3.....	4,080 colonies	22,600 colonies.
No. 4.....	2,472 “
No. 5.....	60,000 colonies	190,000 “
No. 6.....	668 “	2,400 “
No. 7.....	11,340 “

Glucose Peptone Water Fermentation Tubes.

	Reaction.	Gas.	Per Cent.	Proportion.
No. 1-1/200 c.c..... Colon bacilli isolated.	Acid.	+	35	30% CO ₂ H present.
No. 2-1/100 c.c..... Colon bacilli not isolated.	“	+	25	35% CO ₂ H present.
No. 3-1/100 c.c..... Colon bacilli isolated.	“	+	5	30% CO ₂ H present.
No. 4-1/100 c.c..... Colon bacilli isolated.	“	+	20	20% CO ₂ H present.
No. 5-1/200 c.c..... Colon bacilli isolated.	“	+	30	30% CO ₂ H present.
No. 6-1/100 c.c..... Colon bacilli isolated.	“	+	25	35% CO ₂ H present.
No. 7-1/50 c.c..... Colon bacilli isolated.	“	+	20	20% CO ₂ H present.

In addition to the analyses given above, examinations were made of 17 specimens of filtered Croton water from different hospitals, 36 specimens of Croton tap water from different parts of the city and 39 specimens of water from Brooklyn.

METHODS EMPLOYED IN DISINFECTION.

ROBERT J. WILSON, M. D.,

Assistant Director.

For house disinfection formaldehyde has been used to the exclusion of all other gases. In most instances it has been generated by pouring a mixture of 40 per cent. formaldehyde and aluminum sulphate on quick lime.

This method, which has been under observation for two years has given satisfaction. To get uniform results with certainty, it is necessary that the materials used must be selected by competent persons, and that the preparation of the formaldehyde mixture be under the direction of a skilled assistant.

In this department every consignment of formaldehyde solution is tested for its formaldehyde strength.

Before the solution and lime are sent to the disinfectors they are thoroughly tested, and an experimental mixture made; if there is evidence of formaldehyde or its polymers left in the materials they are not put out.

Frequent inspections are made both during and immediately after disinfection for the purpose of examination of the materials used.

The most valid objection to this method is the fact, that in the event of poor materials or the wrong proportions being used there is polymerization of the formaldehyde, and it is not available as a disinfectant. Acrose seems to be the most frequent product of polymerization when the method is used, and as it gives a red color to the lime, the intensity of this color is an indicator of how much gas has been polymerized in the mixture. If the disinfection process has been successful the slacked lime will be pure white.

The bacteriological testing of disinfection in rooms from which goods are to be removed has been made general, and the results shown by the tests are gratifying. In addition to this the card, with its information, to which the test thread was attached, is filed in a card index and can be referred to at any time if it is necessary to determine at what time the room was opened or at what particular hour of the day the disinfection took place.

The general disinfection at the contagious disease hospitals, the ambulance and disinfecting stations and the conveyances carrying contagious diseases is the same as detailed in my last report. At Riverside Hospital the laundry, which was formerly sterilized by passing through a high-temperature water seal, is now sterilized in the autoclave at the disinfecting station of the hospital.

METHODS EMPLOYED IN CLEANING PUBLIC SCHOOLS, STREET CARS, ETC., IN NEW YORK CITY.

In schools the nature of the cleaning is for the most part uniform. After the classes have been dismissed for the day the windows are opened and the cleaners proceed to sweep the rooms with ordinary brooms, no special attempt being made to keep the dust down. There are exceptions to this in a few instances where damp sawdust is used or the floors sprinkled with water. In the morning before the children assemble the rooms are dusted; the teachers' desk and chair and the woodwork of the walls being wiped with a cloth duster, the children's desks and seats being dusted with a feather duster.

Twice a year the school rooms are thoroughly scrubbed with soda solution.

In theatres ordinary brooms were used for sweeping between the seats, and hair brooms on the smooth floors. In some instances during the sweeping process all of the seats were covered over with special covers, which were removed after the cleaning process was over. The woodwork was wiped with cloth dusters, while feather dusters were used for the walls and places not easily reached.

Street cars were cleaned under a system which is about as follows: When the car enters the building where it is to be cleaned, it is immediately visited by the cleaners who remove the mats and sweep the car. As soon as the car is swept the cleaners wash the windows inside and out, following which all of the woodwork of the car is dusted with cloth dusters, waste being used for this purpose.

In tenement houses the hallways were swept with ordinary brooms, and in about nine-tenths of the cases, feather dusters were used in dusting. Where cloth dusters were used it was generally the habit of

the cleaners to use the same duster for all floors; this duster was used dry and shaken from the windows as the cleaners passed from floor to floor. There was a very great accumulation of dust and dirt in the hallways of some of these houses, having its source in the rooms of the tenants, who swept the dirt from their rooms directly into the main hall.

In the better class of apartments, sweeping was done with hair brooms, and the stairs and floors were washed daily with either soda or soap solution. Cloth dusters were used to wipe the woodwork.

Two club houses were visited; in one instance sweeping was done with ordinary brooms and dusting with feather dusters, in the other a combination of ordinary and hair brooms and a sweeping machine was used for sweeping, and the furniture and woodwork was wiped with damp cloths.

In the hospitals visited, sweeping was done with hair brooms, some of which were covered with cheese cloth, dusting was done with cloth dusters.

Railroad coaches were dry swept with ordinary brooms; following sweeping, the windows were cleaned inside and out. Cloth dusters were used to dust the woodwork.

In sleeping cars all of the removable upholstering was taken from the car to a special platform where it was thoroughly cleaned by compressed air. The interior of the car was swept with ordinary brooms and scrubbed with soap solution, the woodwork was wiped over with a special dressing material to keep its appearance good.

The floors of ferryboats were generally wiped with wet mops, and the dusting was done with a combination of cloth and feather dusters. Ferry houses were swept with ordinary brooms, in some instances the floors being first covered with wet saw-dust and in others sprinkled with water, in dusting both cloth and feather dusters were used.

In churches before cleaning was commenced the cushions on the seats were turned upside down. Sweeping was done with ordinary brooms, hair brooms and sweeping machines. The dusting was done with feather dusters and damp cloths and dry cloths.

VIABILITY OF TUBERCLE BACILLI IN DRIED SPUTUM.

BY ANNA I. VON SHOLLY, M. D.,

Assistant Bacteriologist, Research Laboratory.

With fresh sputum proved by microscopical examination to contain a very large number of tubercle bacilli, a test was made to determine their viability when the sputum was dried in the dark and in diffuse daylight. Glass and gauze were the media inoculated with the sputum.

As controls, five healthy guinea pigs, each about 250 gms. in weight, were inoculated with the fresh sputum, four intraperitoneally with 1/100 c.c., 1/1,000 c.c., 1/10,000 c.c., 1/100,000 c.c., respectively, and one subcutaneously with 1/1,000 c.c. Successive batches of guinea pigs of uniform medium size were then inoculated intraperitoneally with emulsions made from this sputum dried at room temperature, one series dried in diffuse light and one in the dark, for 24 hours, 3 days, 9 days, 16 days, 34 and 35 days and 62 and 66 days respectively. The pigs that did not die were killed and autopsied after 72 to 150 days, and their lesions noted. As the time of drying became greater the dose given the animals was progressively increased up through 1/100 c.c., 1/10 c.c., 1/2 and 1 c.c.

Of the five original control animals, two killed after 107 days, showed typical lesions and tubercle bacilli; the two which died after 23 days did not present typical tuberculosis nor were bacilli found. One of these latter was the one inoculated subcutaneously and the other had the smallest dose, viz.: 1/100,000 c.c. One pig disappeared. Of the 3 pigs inoculated after 24 hours drying on glass, from the one dying at end of 34 days from 1/100 c.c. dose, tubercle bacilli were recovered. Two died of accident and cannot be considered in the experiment. Three pigs were inoculated with the sputum dried on gauze for three days in the dark. Two of these receiving 1/100 c.c. and 1/1,000 c.c., respectively, showed tuberculous lesions at autopsy, which were confirmed by finding the bacilli. The pig receiving 1/10,000 c.c. was negative.

At the same time three pigs were injected with emulsions from impregnated gauze dried three days in the light, but one pig disappeared and two met with accidents again. Four of the six pigs inoculated with emulsions from gauze dried 9 days (3 bits of gauze dried in dark and 3 dried in light) showed tuberculous lesions confirmed bacteriologically. Two pigs died of accident, one from each series dried in light and dark.

Of the nine pigs inoculated after 16 days drying in gauze in the dark and in the light, and on glass in the light, 5 showed lesions which were corroborated and 4 did not. These were all killed on 106th to 131st day. Of the 5 tuberculous pigs, 1 was given 1/100 c.c. sputum dried on gauze in the dark, one 1/2 c.c. dried in the dark on gauze, one 1/2 c.c. dried in gauze in the light, and two 1/100 c.c. and 1/2 c.c. each of sputum dried on glass in the light.

None of the pigs injected on the 34th, 35th 62d and 66th days with sputum dried either on glass or gauze, showed tuberculous lesions.

	Fresh Sputum.	Sputum Dried 24 Hours.	Sputum Dried 3 Days.	Sputum Dried 9 Days.	Sputum Dried 16 Days.	Sputum Dried 34-35 Days.	Sputum Dried 62-66 Days.
Number of pigs inoculated.....	4	1	3	6	9	8	5
Pigs showing tuberculous lesions.....	2 killed in 107 days.	1 died in 34 days.	2 { 1 died in 32 days. 1 killed in 72 days.	4 { 1 died in 24 days. 3 killed in 104 days.	{ 5 killed in 106 to 130 days.	{ 8 killed in 113 to 151 days.	0
Pigs showing no tuberculous lesions.....	2 died in 23 days.	0	1 killed in 145 days.	2 { 1 died in 4 days. 1 died in 5 days.	{ 4 killed in 106 to 131 days.	{ 5 killed in 117 to 123 days.	

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FROM THE

RESEARCH LABORATORY

DEPARTMENT OF HEALTH

NEW YORK CITY

Dr. W. H. PARK, Director.

VOLUME II.

1906

DEPARTMENT OF HEALTH

OF THE

CITY OF NEW YORK

DR. THOMAS DARLINGTON

Commissioner of Health

DR. HERMANN M. BIGGS

General Medical Officer

EUGENE W. SCHEFFER

Secretary

In the following pages have been collected all the papers published from the Research Laboratory in the current year, as well as a number of reports and protocols which were unsuited for publication in the regular technical journals. The recipients of this volume will confer a favor on their colleagues of the Research Laboratory, by sending their own publications in exchange. Such pamphlets should be addressed to the Librarian, Research Laboratory, Foot of East Sixteenth street, New York.

THE EDITOR.

New York, December 31, 1906.

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STUDIES FROM THE RESEARCH LABORATORY

For the Year 1906.

THE RESULTS OF THE USE OF REFINED DIPHTHERIA ANTITOXIN, GIBSON'S "GLOBULIN PREPARATION," IN THE TREATMENT OF DIPHTHERIA.

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All who use diphtheria antitoxic serum extensively are aware that in from 10 to 30 per cent. of the injected cases of diphtheria, pronounced rashes, of an urticarial or erythematous type, occur. In the majority of cases these serum effects are disagreeable rather than harmful, but occasionally the rash is accompanied by constitutional disturbance presenting, in the most severe cases, high temperature, vomiting, prostration, and sometimes other symptoms. These marked constitutional reactions are especially likely to follow very large injections of from 10,000 to 20,000 units in young children who have high temperatures due to bronchopneumonia or other complications. In these cases the serum reaction is distinctly harmful, for by lowering the general resistance of the body to other infections it neutralizes to some extent the good done by the neutralization of the diphtheria toxin by the antitoxin. Furthermore, the rashes, especially those of a scarlatinal type, are puzzling in a diagnostic sense.

There have been many attempts made to separate diphtheria antitoxin from the non-antitoxic portions of the accompanying serum. Those interested in the chemical side of these investigations are referred to the recent article by Gibson.* In 1900 Atkinson, working in this laboratory, eliminated all but the globulins from the antitoxic serum. This partially refined antitoxic serum was tried in 36 cases. The results,† both as to antitoxic effect and serum reactions, were so nearly identical with those in an equal number of cases treated with the whole serum from the same horse that it did not seem to be worth while to go to the expense of preparing such an antitoxic solution. Attempts to effect a practical separation of the antitoxin from a greater portion of the proteid non-antitoxic substances of the serum were continued. In August, 1905, we began trials with an antitoxic solution from which much more of the serum proteids had been eliminated than in the Atkinson preparation. Dr. R. B. Gibson, bacteriologist in the Research Laboratory, placed the half-saturation ammonium sulphate precipitate derived from the antitoxic serum in saturated sodium chloride solution, and found that along with a portion of the globulins all the antitoxin passed into solution. In this way the nucleoproteids and the insoluble globulins present in the Atkinson preparation were eliminated. The soluble globulins precipitated by acetic acid were filtered, partially dried, and finally placed in a sac of parchment membrane and dialyzed in running water. This antitoxic solution of soluble globulins was then rendered neutral, and sufficient sodium chloride was added to make it isotonic.

In carrying out the process there is a loss of about 30 per cent. of antitoxin units, because of retention upon filters, loss in dialyzing, etc. On testing this solution on a number of children we found that the results were favorable, except that more local pain was produced than with the whole serum. Stricter attention to the neutralization soon overcame this, so that when the serum was injected on one side and the globulin solution on the other the patient was unable to tell the one from the other. In October, 1905, the antitoxic globulin solution was administered by the medical inspectors not only in the hos-

* Journal of Biological Chemistry, vol. i, Nos. 2 and 3.

† Archives of Pediatrics, November, 1900.

pitals for diphtheria but also in private homes. Since December, 1905, it has been gradually distributed throughout New York City, and is now the only form of antitoxin supplied by the Health Department.

Results from the Use of Antitoxic Globulin Solution—The antitoxic effect was identical with that of the whole serum. Our tests have shown that not only the toxins and the so-called toxones produced in media by diphtheria bacilli, but also those produced in the animal by injections with living diphtheria bacilli are neutralized completely by the globulin solution. We could not detect the slightest evidence that any desirable substance in the antitoxic serum is lost by the refining process. Not only we ourselves, but the resident and attending physicians watching the cases in the contagious disease hospitals noted that the rashes following the injections of the globulin solution seemed to be less severe than those which followed the injection of whole serum. It was especially noted that there were very few who had any constitutional disturbances even when the rashes did appear.

As the serum supplied by different horses, or from the same horse at different times, is known to vary in the rashes and other after-effects it produces, and as it is, therefore, difficult accurately to compare the globulin solution and the whole serum derived from different bleedings, it was decided to make a decisive test by collecting a quantity of serum from four different horses, mixing it thoroughly, and then after precipitating one-half, to treat an equal number of patients simultaneously with the whole serum and with the globulin solution. These tests were carried out chiefly in the Willard Parker Hospital, but a few also in the Riverside Hospital. We are indebted to Drs. Lynah and Watson, the resident physicians in charge of these two hospitals, for their interest and aid.

It soon became evident that the serum we had chosen for the test was of such a character that eruptions and constitutional disturbances usually appeared in those injected with the whole serum. Whether it was because the serum from four long-treated horses had been mixed, or whether because of some other reason, it is certain that this serum produced more after-effects than any lot we had used in the hospital since 1899. These after-effects were so marked and occurred in such a large proportion of the children that we had to abandon the use of

the whole serum. The rashes in those given the globulin preparation were much less severe. In persons over ten years of age almost no rashes occurred after either preparation. The patients treated with the whole serum and the antitoxic globulins were most carefully watched by us and the course of the disease, as well as the after-effects, noted.

TABLE I.

Results of Injecting the Mixed Antitoxic Horse Serum in Fifty Cases of Diphtheria Occurring in Children under Ten Years of Age.

Case.	Age in Years.	Antitoxin Units Given.	Deleterious Effects.	
			Constitutional Disturbances.	Rash.
1 X	0.9	10,000 M	Marked, 5° rise of temperature.	Eighth day, general erythema lasting six days.
2	1	3,000 S	Slight.	Tenth day, urticaria general.
3	1	14,000 M	High temperature, due partly to pneumonia.	Fourteenth day, urticaria lasting five days.
4 I	1.3	15,000 M	Moderate, 2° rise of temperature.	Eighth day, general erythema.
5 X	1.9	5,000 S	
6 I	1.5	35,000 S	High temperature and marked disturbance.	Sixth day, urticaria; eighth day, general erythema of very severe type.
7 X	1.3	10,000 M	
8 X	1.3	13,500 M	Moderate, with 1° rise of temperature.	Fourth day, severe general urticaria lasting three days.
9 X	1.6	10,000 M	Masked by pneumonia having 106° temperature.	Seventh day, severe general erythema.
10	1.7	10,000 M	Moderate, with 2° rise of temperature.	Sixth day, severe general erythema lasting three days.
11 X	1.9	10,000 M	Marked, with 3° rise of temperature.	Third day, morbilliform; eleventh day, severe general urticaria.
12	1.5	7,000 M	Slight.	Fourteenth day, erythema and urticaria general for two days.
13 X	1.5	10,000 S	Slight, with 1.5° rise of temperature.	Second day, quite severe erythema lasting one day.
14 X	2	17,000 M	Slight, with 1.5° rise of temperature.	Third day, very severe urticaria for two days.
15 X	2	10,000 M	Marked, but possibly due to sepsis.	Third day, urticaria and erythema very severe, lasting fifteen days.

Case.	Age in Years.	Antitoxia Units Given.	Deleterious Effects.	
			Constitutional Disturbances.	Rash.
16	2	3,500 S	
17	I	7,000 M	Moderate, 2° rise of temperature.	Fifth day, severe urticaria for six days.
18	X	14,000 M	Slight.	Thirteenth day, severe urticaria for three days.
19	X	3,000 S	Slight.	Twelfth day, general urticaria for two days.
20	2	10,000 M	Slight.	Sixth day, general urticaria for three days.
21	1.5	7,000 S	
22	2.5	7,000 S	Extremely severe, 3°-6° for ten days.	Eighth day, morbilliform continued and intense for ten days.
23	2	7,000 S	Extremely severe, 2°-4° for one week.	Fifteenth day, morbilliform continued and intense for eight days.
24	X	8,000 S	Severe, 4° rise of temperature.	Tenth day, erythema for two days; seventeenth, second lasted two days.
25	X	7,000 M	Severe, 4° rise of temperature.	Seventh day, erythema for two days; twelfth day, second lasted five days.
26	2.8	12,500 M	Slight.	Twenty-second day, general erythema.
27	X	10,000 M	Severe, but possibly due to pneumonia.	Fourteenth day, erythema for five days.
28	X	10,000 M	
29	I	8,000 M	None, except 1.50 rise of temperature.	Sixth day, urticaria for two days.
30	X	7,500 M	
31	I	10,000 M	Severe, 5° rise of temperature for one week.	Thirteenth day, severe erythema for one week until death.
32	X	3,000 S	
33	X	14,000 S	
34	3	7,500 S	Severe, for ten days 4° rise of temperature.	Thirteenth day, severe erythema lasting ten days.
35	3	14,000 M	Very severe, with 4°-7° rise of temperature for ten days.	Eighth day, general erythema over whole body for ten days.
36	4	3,750 S	
37	X	10,000 M	Twelfth day, severe erythema.
38	4	10,000 S	
39	X	10,000 M	Moderate, 2° rise of temperature.	Sixth day, general erythema lasting three days.
40	X	5,000 S	Fifth day, general erythema lasting three days.

Case.	Age in Years.	Antitoxin Units Given.	Deleterious Effects.	
			Constitutional Disturbances.	Rash.
41 X	4	10,000 M	Marked, 4°-6° rise of temperature.	Tenth day, very severe, lasting five days until death.
42	4.5	12,000 S	
43	5	10,000 M	Moderate, 3° rise of temperature.	Fifth day, very severe, urticaria, lasting five days.
44 X	6	5,000 S	
45	6	10,000 M	Moderate, 2° rise of temperature.	Sixth day, general erythema lasting three days.
46	7.5	7,500 M	Marked, 4° rise of temperature.	Fifth day, general erythema. Eighth day, general urticaria.
47	9	10,000 S	Slight.	
48 X	8	3,750 S	
49 X	8	3,750 S	
50 X	9	3,750 S	
Total: 50 cases.	3.24 years average.	Average units per case 9,250	M=28 S=22	Thirty-five developed constitutional disturbances.
				Thirty-six developed rashes.

The character of the case is indicated by the following signs :

x=intubated. i=croup. M=marked severity (of which eighteen were intubated).

s=slight severity. . =absent.

TABLE II.

Results of Injecting Refined Antitoxin (Antitoxic Globulins) Made from Serum Obtained from the same Horses and at the same Bleedings as the Antitoxic Serum Used in the Cases Given in Table I.

Case.	Age in Years.	No. Units of Antitoxin.	Deleterious Effects.	
			Constitutional Disturbances.	Rash.
1	0.5	7,000 S	Sixth day, moderate urticaria and erythema lasting four days.
2	0.9 X	15,000 M	Second day, general erythema lasting two days.
3	1 I	10,000 M	Masked by pneumonia.	Ninth day, general erythema lasting five days until death.
4	1.5	5,000 S	
5	1.5 X	12,000 M	Rise of 1° of temperature.	Third day, urticaria for one day.
6	0.3 X	7,000 S	Second day, general erythema for three days.
7	1.5	12,000 M	
8	.4	3,000 S	
9	1.2 X	10,000 M	Tenth day, urticaria lasting four days.
10	1.2	15,000 M	Eighth day, urticaria lasting two days.
11	1.5 X	12,000 M	Eighth day, urticaria, pretty severe, lasting three days.
12	1.3 X	12,000 M	
13	0.9	5,000 M	Fourth day, erythema lasting thirty-six hours.
14	.5	7,000 M	
15	1.5 X	12,000 M	Fifth day, urticaria for one day.
16	2	10,000 M	
17	2 I	12,000 M	
18	2 I	10,000 M	Seventh day, mild urticaria for one day.
19	2 I	12,000 S	
20	2	24,000 M	
21	2 I	7,000 S	
22	2	7,000 S	
23	2 X	10,000 S	Masked by pneumonia.	Thirteenth day, general erythema lasting three days.
24	2	10,000 M	Tenth day, general erythema lasting three days.
25	2.5 X	12,000 M	Rise of 1° of temperature.	Fifth day, urticaria, then erythema—together lasting five days.

Case.	Age in Years.	No. Units of Antitoxin.	Deleterious Effects.	
			Constitutional Disturbances.	Rash.
26	2.5 X	12,000 M	Rise of 3° of temperature for twelve hours, then normal.	Seventh day, urticaria for two days.
27	3 X	17,000 M	
28	3 X	10,000 M	Eleventh day, erythema for two days.
29	3 I	12,000 M	
30	3 X	20,000 M	Sixth day, urticaria lasting two days.
31	3 X	12,000 M	Second day, slight general erythema lasting twenty-four hours.
32	4	7,000 S	Sixth day, urticaria lasting two days
33	4.5 X	8,000 M	
34	4 I	12,000 S	
35	4.5 I	12,000 M	
36	4 X	12,000 M	
37	4	12,000 M	Rise of 2° of temperature for one day.	Eighth day, severe urticaria, traces lasting five days.
38	4	12,000 S	
39	5	5,000 S	
40	5	12,000 M	
41	5	3,000 S	
42	5	19,000 M	Rise of 2° of temperature for one day.	Sixth day, severe erythema, traces lasting seven days.
43	5.5	3,000 S	
44	6	10,000 S	
45	6	3,000 S	Sixth day, urticaria and erythema for three days.
46	6	4,000 S	Seventh day, urticaria for two days.
47	7 X	12,000 M	
48	8.5 X	24,000 M	
49	9	7,000 S	
50	9	3,500 S	

50	3.18 years average.	10,600 units average injec- tions.	M = 31 S = 19	Constitutional disturb- ances, 5; possibly 7.	Rashes in 23.
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X = intubated. I = croup. M = marked severity. S = slight severity. = absent. Of the fifty cases, there were thirty-one of marked severity; eighteen of these were intubated.

TABLE III.

Comparative Table Giving a Summary of the Constitutional and Local Reactions, Obtained in the Treatment of Fifty Cases of Diphtheria in Young Children with a Lot of Antitoxic Serum Derived from Four Horses and of an Equal Number of Similar Cases Treated with a Solution of the Antitoxic Globulins Derived from a Portion of the Same Lot of Serum.

	Children who were treated with the whole serum.	Children who were treated with the antitoxic globulins.
Marked constitutional symptoms accompanied by severe and persistent rash in.. }	28 per cent.	0 per cent.
Moderate constitutional symptoms accompanied by a well-developed erythema or urticaria..... }	18 "	4 "
Very slight constitutional disturbance accompanied by a more or less general rash.... }	20 "	.. "
No appreciable constitutional disturbance, but a more or less general urticaria or erythema..... }	4 "	34 "
No appreciable after-effects whatever.....	30 "	54 "

TABLE IV.
Duration of Rashes.

	Days.								Total Rashes.
	1	2	3	4	5	6	7	8 and over.	
Antitoxic globulin cases.....	4	7	5	3	3	..	2	23
Whole serum cases.....	1	4	10	1	10	3	2	5	36

After all the tested patients had become fully convalescent or had left the hospital, the histories were finally gone over and compared. It was found that fifty children under ten years of age treated with the whole serum had lived at least nine days, or long enough for the development of serum effects. The first fifty consecutive cases in children under ten years treated with the antitoxic globulins precipitated from the same lot of serum and living nine days or over were taken to compare with these. Table I. gives the salient points for each case treated with the whole serum and Table II. the same for those treated with the solution of antitoxic globulins. Tables III. and IV. summarize these points.

It is noticeable that not only were the rashes more frequent, but also much more persistent in the patients who received the whole serum. Twenty-three rashes following the use of the whole serum lasted over three days in this series, as against only six in the antitoxic globulin cases.

Summary and Conclusions—The results obtained in these series of one hundred cases are so definite that it seems safe to conclude that the removal of a considerable portion of the non-antitoxic globulins, as well as the albumins from the serum by the Gibson method, has eliminated much of the deleterious matter from the serum, so that severe rashes, joint complications, fever, and other constitutional disturbances are less likely to occur from the antitoxic globulins than from the antitoxic serum from which it was obtained. The globulin preparation when tested by animal experiments appears to retain all the antitoxic properties of the whole serum. The portion of the globulins still accompanying the antitoxin in the Gibson preparation is shown to be capable of exciting rashes and occasionally constitutional disturbances, although, as stated above, to a less extent than the serum. It is almost certain that methods will be devised to refine antitoxin still farther, and so possibly eliminate all appreciable deleterious effects of the antitoxic serum.

Whether this globulin solution will be much less likely than the serum to cause collapse in the rare cases of peculiar susceptibility, such as in a certain percentage of those suffering from status lymphaticus, is still undetermined. It has now been used in several thousand cases of diphtheria without accident.

The concentration of antitoxin made possible by the elimination of the non-antitoxic substances is not only a convenience, but of distinct clinical importance, as it tends to encourage large doses.

The antitoxic globulin solution, like the serum, tends to become slightly cloudy when kept at moderate or high temperature, and substances such as solutions of carbolic acid and trikresol are especially likely to cause a precipitate to develop. The antitoxin in the globulin preparation retains its potency about as long as that in the whole serum.

THE ETIOLOGY AND DIAGNOSIS OF HYDROPHOBIA.

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Introduction.

During the spring of 1904 the "Negri bodies" were demonstrated by one of us in smears from the central nervous system of animals dead from hydrophobia. At that time, however, the technic was poor and the stains were unsatisfactory, so the use of the method in diagnosis was not begun. Many of the cases reported by Dr. Poor were studied by us in this way, the "bodies" being demonstrated in smears from three horses and from several dogs and guinea pigs, while they were not found in normal dogs, guinea pigs, or rabbits, or in guinea pigs dead from tetanus or diphtheria toxin.

Last fall, in connection with the study of smears from vaccinia and variola stained by Giemsa's method, smears from hydrophobia cases were again tried and it was found that the "bodies" were brought out very clearly and characteristically by the Giemsa solution; and, as a consequence, the present work was planned.

Some of the most interesting material used by us has been obtained through the kindness of Dr. R. J. Wilson and of a number of veterinarians of New York City, to all of whom we wish to express our thanks. Most of the sections have been prepared by Miss C. R. Gurley. All of the work has been done with the help and encouragement of Dr. Wm. H. Park, to whom thanks are due.

Historical Review.

Investigations on hydrophobia have been carried on from three principal standpoints; first, the therapeutic; second, the diagnostic; and third, the etiologic. Since the establishment of the Pasteur treatment, the importance of making a quick diagnosis has become so evident that the efforts of many workers have been directed toward this end, and only occasionally has the purely etiologic standpoint been considered.

Pasteur and his immediate followers relied for their diagnosis entirely upon rabbit inoculations, and this meant a fifteen to twenty days'

wait before the patient knew whether or not the treatment he was receiving was necessary. In 1898 this time was shortened to about nine days in our laboratory by Wilson, who found that guinea pigs came down with the disease much more quickly than rabbits.

From time to time it has been thought that certain histologic findings were diagnostic; for instance, the "rabid tubercles" of Babes, and the areas of "round and oval-celled accumulation in the cerebrospinal and sympathetic ganglia" of Van Gehuchten and Nelis, were said to be specific, but further study has shown that they are not absolutely specific for rabies. In many cases the whole picture of the grosser histologic changes is sufficiently characteristic to warrant the diagnosis of rabies, but often it is not so. Bailey, in his studies on the ganglion cells in normal and hydrophobic rabbits gives a good bibliography of the histologic findings up to 1901. It was not until Negri, in 1903, described certain bodies seen by him in the large nerve cells in sections of the central nervous system that anything was found which seemed absolutely specific for hydrophobia. Negri claims that these bodies are not only specific for rabies, but that they probably are animal parasites, and the cause of the disease.

He describes them as usually round or oval bodies from $1m$ to $23m$ long, and containing vacuoles in some of which are granules of varying size and number; generally there is a central larger structure surrounded by smaller ones. By Mann's method of staining, the organism generally takes a brilliant eosin-red, with the exception of the granules, some of which stain a light blue, and the others a faint red. The central structure gives the appearance of being a nucleus. The bodies are sometimes in touch with the nucleus of the host cell, sometimes far from it, often in the cell branches where they are more elliptical in shape. There are irregular, pear-shaped, and three-cornered forms, all of which special shapes Negri thinks due to the position of the organism within the host cell. He speaks of multiplying forms but does not describe any definite division forms. He says he is able to identify the bodies in the hanging-drop fresh, and in a weak acetic acid solution; but does not recommend this method for general use in diagnosis, as it is difficult to differentiate the bodies under these conditions from the nerve-tissue elements. He finds his organism generally in greatest

numbers in the large nerve cells of Ammon's horn, less frequently in those of the cerebral cortex, the cerebellum, the medulla, the spinal cord, and the cerebrospinal ganglia. The organisms vary greatly in numbers in the different cases. In some cases he finds only an occasional one, while in others they are innumerable.

He says very little about the bodies in animals dying of fixed virus, merely stating that they have been found in rabbits that have died on the seventh day after inoculation with fixed virus, but that they are very tiny, infrequent, and found with difficulty. He finds the largest forms in dogs inoculated subdurally with street virus.

Negri's work was soon corroborated by many Italian observers. Volpino, Bertarelli, D'Amato, Daddi, Di Vestea, Guarnieri, and Martinotti, published almost immediately after Negri's first publication. They were soon followed by Celli and De Blasi, Pace, and Bosc. The "bodies" have been found by these authors, and later by others in all varieties of animals which are susceptible to hydrophobia, i. e., in dogs, cats, rabbits, rats, mice, guinea pigs, birds, cattle, horses, and human beings.

In 1904 Luzzani published a report of 179 cases, and in all but nine of those which were proven by animal inoculation to be rabies, the "Negri bodies" were found.

In our own laboratory in the same year, Poor examined 19 cases of street rabies and many cases of experimental rabies, and in all except those inoculated with fixed virus the "bodies" were found. In fixed-virus animals he found an occasional homogeneous eosinophilic granule in the cerebellar cells, about which he expressed no opinion. Similar granules were also seen by various other observers, some of whom consider them possibly tiny forms of the organism; but nothing definite has been observed about them, and as indefinite granules have also been seen in other conditions, their significance is uncertain.

In 1904 Negri's work, so far as the presence of these "bodies" in hydrophobia is concerned, was further corroborated by the following workers: Dominici, Marzocchi, Bandini, Fasoli, and Schüder. There was no dissenting voice as to their presence, and as to their diagnostic value. Many controls were made by the different observers, especially by Volpino, Marzocchi, Dominici, and Poor. They examined the cen-

tral nervous system of various animals that had died from poisoning with tetanus, strychnine, pneumococcus staphylococcus, alcohol, formalin, tubercle bacillus, diphtheria toxin; and of human beings who had died from epilepsy, syphilis, alcohol poisoning, tuberculosis, and various nervous affections. Many normal animals were also examined, all with negative results so far as the "Negri bodies" were concerned.

During this time the "bodies" were tested for their resistance to various physical and chemical agents, such as heat, cold, drying, immersion in glycerin, etc., and they were found to retain their characteristic appearance and virulence after more or less manipulation. It does not necessarily follow, however, that the "bodies," even if they are living organisms, need to retain their characteristic appearance in order to be virulent. We know, for instance, that trypanosomes may seem to disappear from blood which continues to be infective. (Laveran and Mesnil.)

Remlinger showed that the medulla of rabbits inoculated subdurally with fixed virus may be virulent on the third day, but he did not work out the exact degree of virulence—that is, the approximate number of organisms in the material inoculated. The fact that it is virulent soon after inoculation, and that no "Negri bodies" have been found at this early period, he thinks is another indication that they are not organisms. He does not consider the possibility of there being tinier forms than those so far seen, but believes that the organism in its whole life cycle is ultra-microscopic in size.

In regard to the significance of the "bodies," up to 1905 all of these authors, with two exceptions, agree with Negri in considering them probably Protozoa and the cause of hydrophobia. The two exceptions are Remlinger and Schüder.

These latter investigators consider the fact that the virus can be filtered through a filter, practically impervious to ordinary bacteria, a proof that the "Negri bodies," which they say are too large to pass such a filter, are not the cause of hydrophobia. Bertarelli, however, showed that the residue after filtration was also virulent, and he and others expressed the opinion that besides forms too large to pass the filter there might be forms tiny enough to do so. We know that in a medium containing a growing protozoon we may find both large and

small forms, the limits in size of the smallest forms not being known in some cases; the fact therefore, that the filtered portion and unfiltered solid residue both possess virulence is an added indication that we are dealing with Protozoa. MacNeal has shown with the trypanosomes that besides the large forms, there are forms tiny enough to pass a Berkefeld.

Practically nothing has been done with regard to the exact degree of virulence possessed by filtered and unfiltered portions of the emulsions of rabies virus. Late in 1905, Di Vestea showed that the filtered virus possesses characteristics different from unfiltered, thus indicating that the forms in each may be different in character. He thinks that the undiscovered extracellular forms may be tiny enough to pass the filter.

Quite recently Volpino elaborates more fully an hypothesis advanced by him in 1904, in regard to the filterable forms. He thinks that the real organism is very tiny, that probably only the inner bodies in the so-called "Negri body"—the tiny bodies which he had shown to be definite basophilic forms—are the parasites, and that the homogeneous-appearing substance in which they are imbedded and which makes up the rest of the "Negri body," as Negri describes it, is derived from the host cell, caused by the reaction of it to the parasite. He gives a number of drawings arranged in the form of a life-cycle to illustrate this idea.

Negri's latest article, appearing in June, 1905, states that the central body shows more characteristically as a nucleus in sections from rabid cattle which he had stained in a special way by hematoxylin, and that in the same animals there appear bodies presenting characteristics of cysts. These later studies confirm all of his previous work and emphasize the fact that some of the bodies contain a central complex characteristic mass of chromatin, sometimes appearing solidly stained, sometimes as a distinct network, and sometimes encircled by smaller solidly staining masses of chromatin. Each chromatin mass is surrounded by a clear, unstained ring.

The bodies which he interprets as cysts, he describes as similar in dimensions, shapes, and general staining characteristics to the other forms, but different in minute structure. By the staining method of

Mann they seem to be filled with tiny, refractive, somewhat elongated granules. Some seem to be surrounded by a membrane which is occasionally notched as if about to break. The iron-hematoxylin stain brings out the structure of these bodies very clearly. They seem to be filled with numerous black-staining "spores" less than 1 micron long and narrower, which appear as tiny filaments slightly curved with a small swelling near the center.

In 1905 still other workers corroborated Negri's work, among them Abba and Bormans, Way, Zaccaria, Maresch, Schiffmann, Galli-Valerio, and Bohne. Only one author failed to corroborate the work. Maas, in sections from a case of human rabies could find no "Negri bodies." Luzzani in this year published another collection of cases. Out of 457, 297 proved by the biologic test to be hydrophobia, and in only nine of these were the "bodies" not found in sections. The bodies were not found in any other animal.

Maresch, by Bielschowsky's staining method, claims to have brought out the structure more distinctly.

Schiffmann, after studying the "Negri bodies" as they appear in street rabies and examining many controls, confirming fully the diagnostic value of the "bodies," studied the changes which they seemed to undergo in passage from animal to animal of the same species and of one species to another. He states that the greater the number of passages through a single species of animal, the smaller the "bodies," until in "fixed virus" in the rabbit no forms appear. He also says that he did not find any "bodies" in dogs inoculated with rabbit-fixed virus.

Bohne describes the shortest method so far published for examination of sections. The whole process lasts only three hours, and the author states that it is very satisfactory. The method is as follows: Small pieces of the nerve tissue are placed in 15 c.c. of pure acetone and kept at 37 degrees C. for about 30 to 45 minutes. They are then put in 55 degrees paraffin and left from 60 to 75 minutes, boxed, cut at 6m, dried at 60 degrees, and stained with a modified Mann's method in 4 minutes. The "bodies" show a vacuolated and granular structure and some of the elliptical forms seem to be dividing. On the whole they

take more of a magenta stain than the "bodies" do in sections prepared in the regular way. The author considers their parasitic nature still doubtful.

During 1905 a good review of recent studies on hydrophobia came out in the "Bulletin de l'Institut Pasteur," and in 1906 Bertarelli published a good review in the "Referate" of the "Centralblatt für Bakteriologie."

We may sum up the results obtained from the foregoing studies as follows:

1. In nearly 100 per cent. of definite cases of street rabies characteristic "bodies" are found in the large nerve cells of sections from all or from a part of the central nervous system and the connected ganglia.

2. The general characteristics of most of these "bodies" are as follows: rounded or oval forms varying in size from $1m$ to $25m$, with a homogeneous acidophilic ground substance containing a central body surrounded by granules; these inner bodies vary in structure and staining qualities, but are principally basophilic and may be in the form of reticular masses, rings, rods, or small granules, they are usually situated within vacuoles.

3. The "bodies" vary also in number, being very few in some cases, and numerous in others. According to one author they become fewer the greater the number of passages through a single species of animal, and are not found in fixed virus. Others have found occasional small forms in fixed virus, but not in large enough numbers to account for the infectivity of the nerve tissue.

4. No "bodies" have been found before the appearance of symptoms, although the central nervous system is infective before this time.

5. No "bodies" have been found in the peripheral nerves or in the salivary or other glands, although these organs have been shown to possess a certain amount of infectivity.

6. The most rapid satisfactory method of demonstrating the "bodies" for diagnosis is a complicated section process which takes at least three hours.

The filtered virus is infective, therefore some forms of the causative agent must be extremely tiny.

8. In no other disease have bodies similar in appearance to the "Negri bodies" been found.

9. When the "bodies" are found in sections, the diagnosis of hydrophobia is certain and the biologic test need not be made; when they are not found, the case may have been one of hydrophobia and the biologic test must be made.

10. The significance of the "bodies" is still in doubt for the following reasons: (a) They have not been found in all cases of hydrophobia, notably not in fixed virus, neither have they been found in all parts of nervous tissue proved to be virulent, especially before the beginning of symptoms; (b) forms small enough to pass the coarser Berkefeld filters have not been seen; (c) the structure has not been shown definitely to be analogous to that of known living organisms; (d) no definite series of forms indicating growth and multiplication has been demonstrated; (e) the staining qualities, contrary to those of known Protozoa, are more acidophilic than basophilic.

In January of 1906 one of the writers made a preliminary communication of part of the work reported in the following pages. Emphasis was placed upon the fact that the demonstration of the "Negri bodies" by the "smear method" which was recommended by this writer in 1904 (see discussion under Poor's first article) had, by better technic, proved to be wonderfully successful. By this method the structure of the "bodies" is brought out more definitely than by the section method, and the whole process is much simplified and may be completed within half an hour after removal of the nerve tissue from the animal.

The method of examining the central nervous system, especially the brain, by smears has been used by several pathologists, among whom may be mentioned Ewing, who obtained interesting results by this method in his studies on the pathology of ganglion cells.*

* Just after this paper went to press the article by Frothingham appeared. His work corroborates the results obtained by the smear method of diagnosing rabies. We have tried the impression method which he describes, as well as a number of other methods of making smears of the central nervous system and find the results obtained by them all good in some particulars, but the method we describe has so far given us uniformly better results in the diagnosis work.

Original Work.

The work may be divided into two parts:

I. The value of the "Negri bodies" in diagnosis and their rapid identification.

II. A study of the "bodies" with a view to determining their nature.

In all, 141 animals, including seven different species, have been studied with these two points in view. The following table gives a classified list of these animals:

Street rabies cases	{ Dogs	25
	{ Cat	1
	{ Human beings.....	3
Animals inoculated with street rabies	{ Dogs	7
	{ Rabbits	12
	{ Guinea-pigs	32
	{ Mice	5
Animals inoculated with fixed virus	{ Dog	1
	{ Rabbits	27
	{ Guinea-pigs	7
	{ Mice	1
Control animals	{ Dogs	12
	{ Rabbits	4
	{ Guinea-pigs	2
	{ Calf	1
	{ Human being.....	1

I.

In the first part of the work we have tried to determine: (1) Whether the "bodies" seen in the smears are similar to those seen in the sections, (2) the correspondence between the smear method, the section method, and the biologic test, (3) the comparative value of each method in diagnosis, and (4) the specificity of the "bodies."

It was decided that these points might be brought out by using all three diagnostic tests in a series of street rabies animals and of a number of controls. Therefore with each animal chosen for this purpose the following routine was carried out: (1) the brain, medulla, and parts of the spinal cord and connected ganglia were removed; (2)

small pieces from each part were fixed in Zenker's fluid; (3) smears were made from corresponding parts; and (4) animals were inoculated subdurally with an emulsion of corresponding parts, and from the animals that died either smears or sections or both were made.

The technic of the smear work is as follows:

1. Glass slides and cover-glasses are washed thoroughly with soap and water, then heated in the flame to get rid of oily substances.

2. A small bit of the gray substance of brain chosen for examination is cut out with a small sharp pair of scissors and placed about one inch from the end of the slide, so as to leave enough room for a label. The cut in the brain should be made at right angles to its surface and a thin slice taken, avoiding the white matter as much as possible.

3. A cover-slip placed over the piece of tissue is pressed upon it until it is spread out in a moderately thin layer, then the cover-slip is moved slowly and evenly over the slide to the end opposite the label. Only slight pressure should be used in making the smear, but slightly more should be exerted on the cover-glass toward the label side of the slide, thus allowing more of the nerve tissue to be carried farther down the smear and producing more well-spread nerve cells. If any thick places are left at the edge of the smear, one or two of them may be spread out toward the side of the slide with the edge of the cover-glass. If the first smear does not seem to be well spread out others should be made until a satisfactory one is obtained.

4. For diagnosis work such a smear should be made from at least three different parts of gray matter of the central nervous system; first, from the cortex in the region of the fissure of Rolando or in the region corresponding to it (in the dog the convolution around the crucial sulcus), second, from Ammon's horn, third, from the cerebellum. In many of the animals reported here, smears were made from the gray matter of the cerebral cortex, around the fissures of Rolando and Sylvius, from the olfactory nerves, spinal cord in the dorsal and lumbar regions, spinal and Gasserian ganglia, salivary glands, suprarenals, pancreas, and some of the peripheral nerves. From the last four-named structures the smears were not very successful, so only a few were made.

5. The smears are dried in air,[†] and subjected to one or both of the two following staining methods:

(a) Giemsa's solution. The smears are fixed in methyl alcohol (commercial is just as good as pure) for about 5 minutes. The staining solution recommended last by Giemsa[‡] (1 drop of the stain to every c.c. of distilled water made alkaline by the previous addition of one drop of a 1 per cent. solution of potassium carbonate to 10 c.c. of the water) is poured over the slide and allowed to stand for one-half to three hours. The longer time brings out the structure better, and in 24 hours well-made smears are not overstained. After the stain is poured off, the smear is washed in running tap water for one to three minutes, and dried with filter paper. If the smear is thick, the "bodies" may come out a little more clearly by dipping in 50 per cent. methyl alcohol before washing in water, then the washing need not be as thorough. By this method of staining, the cytoplasm of the "bodies" stains blue and the central bodies and chromatoid granules stain a blue-red or azur. Generally the larger "bodies" are a darker blue than the smaller, the smallest of all may be very light (Plate 19, Journ. Infect. Diseases, 1906). The stain varies somewhat according to the thick-

[†] This method has proved so practical in our hands that an effort is being made to extend its usefulness.

The Board of Health of New York City has prepared a circular containing a description of the foregoing technic with more explicit directions in regard to the regions from which the smears are to be made with the added information that such smears, as well as the fresh material, may be sent to the nearest laboratory familiar with the appearance of the "Negri bodies" or to the Research Laboratory of the N. Y. Health Department. If the smears have been made successfully and the "Negri bodies" are found, the sender may receive word almost immediately and no sections or inoculations of the material need be made.

‡Azur II—Eosin.....	3.0 g.
Azur II.....	0.8
Glycerin (Merck. chem. pure).....	250.0 c.c.
Methyl alcohol (chem. pure).....	250.0

Both glycerin and alcohol are heated to 60° C. The dyes are put into the alcohol and the glycerin is added slowly, stirring. The mixture is allowed to stand at room temperature over night, and after filtration is ready for use.

The solution is prepared ready for use by Grüber, Leipzig.

ness of the smear. Some have a robin's-egg blue tint but this is after a longer fixation in the methyl alcohol. In this case the red blood cells may have a greenish tint. (See Part II. for full description of "bodies" stained by this method). The cytoplasm of the nerve cells stains blue also, but with a successfully made smear the cytoplasm is so spread out that the outline and structure of most of the "bodies" are seen distinctly within it. The nuclei of the nerve cells are stained red with the azur, the nucleoli a dull blue, the red blood cells a pink-yellow, more pink if the decolorization is used. The "bodies" have an appearance of depth, due to their refractive qualities.

For diagnostic purposes this method of staining may be shortened as follows: Methyl alcohol, 5 minutes, equal parts of the Giemsa solution and distilled water, 10 minutes. In this way "bodies" are generally brought out well enough for diagnosis, and sometimes the structure shows distinctly. It is always well, however, to make smears enough for the longer method of staining, in case the shorter one should prove unsatisfactory.

(b) The eosin-methylene blue method recommended by Mallory. The smears are fixed in Zenker's solution for one-half hour; then after being rinsed in tap water they are placed successively in 95 per cent. alcohol + iodine one-quarter hour, 95 per cent. alcohol one-half hour, absolute alcohol one-half hour, eosin solution 20 minutes, tap water for rinsing, methylene-blue solution 15 minutes, 95 per cent. alcohol for differentiation lasting from one to five minutes; after this they are blotted with fine paper and allowed to dry. With this method of staining, the cytoplasm of the "bodies" is a magenta, light in the small bodies, darker in the larger; the central bodies and chromatoid granules are a very dark blue; the nerve cell cytoplasm is a light blue; the nucleus is a darker blue, and the red blood cells are a brilliant eosin pink. With more decolorization in the alcohol the "bodies" are not such a deep magenta and the difference in color between them and the red blood cells is not so marked.

The "bodies" and the structure are often more clearly defined with this method and perhaps on the whole it is better to use it for making diagnosis: but when there are only tiny "bodies" present, or when the

brain tissue is old and soft, the Giemsa stain seems to be the more successful; above all, when one wishes to study the nature of the central structures and granules the Giemsa stain must be used. We therefore recommend strongly the use of both methods. Even if both are used and one has to wait for the longer method, the technic is far simpler than any so far published. §§

Not only do the "bodies" come out more distinctly by the smear method, but the pathological changes accompanying them are well demonstrated. For instance, the swellings of the neuro-fibrils described by Ramon y Cajal, the collections of the lymphoid cells, the increase of the endothelioid cells, the degenerated nerve cells are all clearly seen.

The technic of the section work is as follows: (1) The small pieces are left in Zenker's fluid for three to four hours; (2) washed in tap water for five minutes; (3) placed in 80 per cent. alcohol+iodine (enough tincture of iodine added to give port wine color) for about 24 hours; (4) 95 per cent. alcohol+iodine 24 hours; (5) 95 per cent. alcohol 24 hours; (6) absolute alcohol from four to six hours; (7) cedar oil until cleared; (8) cedar oil+paraffin 52 degrees C., two hours; (9) paraffin 52 degrees two hours in each of two baths; (10) boxing; (11) sections are cut at 3 to 6m, dried in thermostat at 36 degrees C. for about 24 hours protected from the dust, and stained according to the eosin and methylene blue method recommended by Mallory. The most important point in the technic is the time the material is allowed to remain in Zenker. According to our experience, two hours fixation

§§ Van Gieson working in our laboratory, suggests a staining method which differentiates the "Negri bodies" more quickly than either of the two methods described above. So far, the best proportion of the stains used have not been determined, but satisfactory results have been obtained from the following mixture: To 10 drops of distilled water three drops of a sat. alc. sol. of rose aniline violet and six drops of Loeffler's solution of methylene blue are added. The smears are fixed while moist, in methyl alcohol for one minute. The stain is then poured on, warmed until it steams, poured off, and the smear is rinsed in water and allowed to dry.

The cytoplasm of the "bodies" is a deep and distinctive red or magenta, their inner structures are a dark blue, the nerve cells are a light blue and the blood cells a pale salmon-red.

The staining mixture remains good for about an hour.

is not enough, three to four hours is very good, and with every hour after five hours the results become less satisfactory. Left in Zenker over night the tissue is granular and takes the eosin stain more or less deeply, both of which results interfere with the appearance of the tiniest "bodies," especially of the very delicate, tiny forms found by us in sections from fixed virus. Another point in favor of the short fixation in Zenker is that the precipitate formed by the mercury is not so great and is more easily got rid of, which is a very great help in the identification of the tiniest forms. Schiffmann recommends short fixation in Zenker, but he does not state the time he finds best.

It is thought, also, that washing for any great length of time in water after fixation does not help the specimens; the few that were left for a much longer time than the five minutes are not as satisfactory as the others.

In regard to the rest of the technic, it is sufficient to say that the changes to the different fluids were made with great regularity, and the final differentiation in alcohol of the stained sections was done most carefully.

In the sections made in this way we have been able to demonstrate clearly very tiny forms as well as good structure in the larger forms, a description of which will be given in Part II.

TABLE I.

Results of Examination of Rabies Material by Means of Smears, Sections and Animal Inoculations.

Number.	Species.	Date of Autopsy.	Clinical Diagnosis.	Presence of Negri Bodies in Smears.	Presence of Negri Bodies in Sections.	Result of Animal Inoculation.	Presence of Negri Bodies in Smears from Animals Inoculated.	Presence of Negri Bodies in Sections from Animals Inoculated.
1..	Dog	1905. 11-10	Rabies	+	::	+	::	::
2..	"	11-23	Rabies	+	+	::	::	::
3..	"	12- 2	Doubtful	+	+	::	::	::
4..	"	12- 4	Rabies	+	+	::	::	::
5..	"	12- 9	Suspicious	-	-	-	::	::
6..	"	12- 9	Suspicious	-	-	-	::	::
7..	"	12- 9	Suspicious	-	-	-	::	::
8..	"	12-15	Rabies	+	::	+	+	+
9..	Dog	1906. 1- 4	Rabies	+	+	+	+	+
10..	"	1-10	Rabies	+	+	::	::	::
11..	"	1-18	Doubtful	-	::	-	::	::
12..	"	1-22	Rabies	+	+	+	+	+
13..	"	1-26	Rabies	+	+	+	+	+
14..	"	1-29	Rabies	+	+	::	::	::
15..	"	2-20	Rabies	+	+	+	+	::
16..	"	2-23	Rabies	+	+	+	+	+
17..	"	2-26	Rabies	D'tful*	+†	+	+	+
18..	"	2-26	Doubtful	-	-	-	::	::
19..	"	2-27	Rabies	+	::	+	::	::
20..	"	3- 2	Rabies	+	+	+	+	+
21..	"	3- 3	Rabies	+	+	+	+	+
22..	"	3- 6	Distemper or rabies	+	+	::	::	::
23..	"	3-12	Rabies	+	+	+	+	+
24..	"	3-13	Rabies	+	+	+	+	+
25..	"	3-26	Rabies	+	+	::	::	::
26..	Cat	1905. 12- 5	Rabies	+	::	+	::	::
27..	Human	11-10	Rabies	+	+	+	+	::
28..	Child	11-16	Rabies	+	+	+	+	::
29..	Child	1906. 1-16	Rabies	+	+	+	+	+

* Brain in bad condition; two days old.

† A few tiny "bodies" found.

TABLE I (Continued).

Number.	Species.	Date of Autopsy.	Clinical Diagnosis.	Presence of Negri Bodies in Smears.	Presence of Negri Bodies in Sections.	Result of Animal Inoculation.	Presence of Negri Bodies in Smears from Animals Inoculated.	Presence of Negri Bodies in Sections from Animals Inoculated.
30..	Human	1-9	Alcoholic neuritis.....	-	-	-
31..	Dog	1-4	{ Inoculated with human rabies; no symptoms.....	-	:	:	..	:
32..	"	1-16	{ Inoculated with human rabies; no symptoms.....	-	-	:	..	:
33..	"	1-30	{ Inoculated with human rabies; typical symptoms.....	+	+	:	..	:
34..	"	1-31	{ Inoculated with human rabies; typical symptoms.....	+	+	+	..	:
35..	"	2-6	{ Inoculated with human rabies; typical symptoms.....	+	+	+	..	:
36..	"	2-15	{ Inoculated with human rabies; typical symptoms.....	+	+	+	+	+
37..	"	3-6	{ Inoculated with street rabies; typical symptoms.....	+	..	+	+	+
38..	Calf	1905. 11-5	Normal.....	-
39..	Dog	11-13	Normal.....	-
40..	"	11-14	Normal.....	-
41..	"	11-14	Normal.....	-
42..	"	12-1	Normal.....	-
43..	"	12-1	Normal.....	-
44..	"	Normal.....	-	-	-

In Table I we have given the results of the animals studied with a view of determining the four points mentioned at the beginning of this section. In some of them the full examination as planned was carried out, in others, besides the smears, only sections or animal inoculations were made. The controls are not as many as we might have made had not so much control work been done previously by us and by so many others.

The results are as follows:

I. No control animal shows appearances similar to the "Negri bodies," either in smears or in sections. The various suspicious cases, especially the case of the dog with filaria, we consider among the best controls, because here we are dealing with animals dead after symptoms similar to those of hydrophobia.

2. In all of the cases proved by the biologic test to be hydrophobia, "Negri bodies" are found in either smears or sections or in both.

3. In the animals which had been inoculated from these animals, "Negri bodies" are found in either smears or sections or in both.

4. The general characteristics of the "bodies" seen in the smears are similar to those of the "bodies" seen in sections.

5. The three tests correspond as to diagnostic results.

6. The smear method is much better than the section method in demonstrating the "bodies" for diagnostic purposes.

7. When the "bodies" are present in the smears the diagnosis of hydrophobia is certain, even if the biological test is negative. When they are not found the diagnosis is uncertain.

8. In a very few cases of street rabies, only extremely tiny forms are found. These may be easier to find in sections than in smears.

9. In doubtful or negative cases both the section method and animal inoculations should be tried.

In studying the nature of these bodies many points have only been touched upon and others are still being investigated, but we believe that enough new knowledge has been gained to warrant this publication.* The plan of this part of the work is as follows:

1. The comparison of the general characteristics of the "bodies"

in smears.
in sections.
in hanging-drop.

 - (a) Size

in different species of animals.
in different animals of same species.
in different parts of same animal.
in different stages of the disease.
in different numbers of passages.
after different modes of inoculation.
 - (b) Shape
 - (c) Number
 - (d) Site
 - (e) Structure
2. Detailed characteristics of structure.
 - (a) Cytoplasm.
 - (b) Central bodies.

*[The Editor regrets being unable to reproduce the plates accompanying the original article. They will be found on page 483 of the "Journal of Infectious Diseases," Chicago, 1906.]

- (c) Chromatoid granules.
 - (d) Different shapes.
 - (e) Division forms $\left\{ \begin{array}{l} \text{transverse.} \\ \text{longitudinal.} \\ \text{budding.} \end{array} \right.$
 - (f) Conjugation forms.
 - (g) Stages at which different forms appear.
3. Relation between the time the central nervous tissue becomes infected and the time the bodies appear.
 4. Spread of the bodies to different parts of the host.
 5. Significance of the bodies and comparison with known organisms.
 6. Summary.

1. *General Characteristics of Bodies in Smears Compared with those in Sections: Size.*—The majority of the forms seem larger in smears than they do in sections from the same case. The largest forms measured are about 18 *m* and the smallest structured forms about 0.5 *m*. We can easily see that a form appearing as 0.5 *m* in a smear might scarcely be visible in a section, and that such tiny forms, considering their extreme plasticity (see under structure), might easily pass the coarser Berkefeld filters. We have found that the size varies more with the course of the disease (which includes the question of accustoming the virus to the host (e. g. fixed virus), than it does merely with different species of animals. This means that the bodies may vary greatly in different animals of the same species, in different parts of the same animal, and at different stages of the disease. We may say in general that no very large forms are found in the early stages of the disease or in any stage in certain varieties of especially susceptible animals to which the virus has become accustomed (fixed virus). While in later stages of the disease in animals inoculated with virus from another species, or in varieties of animals that are not fully susceptible to the disease, both large and small forms are found.

We have not yet had the opportunity of examining smears from rabid cattle, so we are not able to corroborate the statement of Negri that the largest forms are found in this variety of animal; but if it holds, it would seem that the reasons for the fact might be that cattle

are among the less susceptible animals, and that they are generally inoculated with a virus from a different species of animal. Of course, other things being equal, we should expect a certain amount of variation in size and structure of an organism growing in different species of animals, just as we get variations in the same variety of bacteria and of other low forms of life grown in different culture media.

Shape—The shape of the bodies appears more varied in smears than in sections, due partly to the fact that there is a certain amount of distortion. The distortion, however, is very slight, because within narrow limits of disturbance (i. e., too much or too unequal pressure in making the smears) the bodies are broken up and their identity lost. The principal types of shapes seen in smears are given in Plate 19, Figs. 3 to 56 inclusive, "Journal of Infectious Diseases," 1906, p. 484. The same types of shapes are seen in all varieties of animals studied.

Number.—Generally more bodies are seen in smears than in sections from similar parts of the same case. Since we have learned to identify many tiny bodies, we have found that there are more in all cases, including fixed-virus cases, than have hitherto been reported. In any case we feel that we are able to demonstrate enough forms, or, at least, to account for enough forms, to correspond to the degree of infectivity of the part.

Site.—As is shown in Plate 18, Fig. 2, in the "Journal of Infectious Diseases," 1906, page 484, the topography of the bodies may be well preserved in smears. Their situation in the cytoplasm of the body and branches of the larger nerve cells is well shown. In parts of the smear which are more broken up the bodies may appear as if lying free, and it is these bodies, if the pressure has not been too great, that show the structure best. Such bodies have for the most part been chosen for the photographs (especially 1, 2, 4 and 5). There are often many tiny "bodies" in degenerating nerve cells, but these show better in sections than in smears. The tiny forms which we have seen in the nuclei of the host cells also appear more distinct in sections than in smears.*

* With Van Gieson's new staining method these tiny forms are better differentiated in smears.

Structure—The principal point in favor of the smear method of examination is that the structure of the bodies comes out so clearly and so characteristically that it is easy to draw a close analogy between it and that of known Protozoa. In the first place, as has been shown by Negri and most of the other investigators, the following fact holds true: Whatever the variety or species of animal infected, the bodies preserve their same general characteristic structure, i. e., a hyalin cytoplasm with an entire margin, and with one or more inner bodies having a more or less complicated and regular structure. This fact alone, that by such an entirely different method of examination the bodies show the same characteristic structure in so many different varieties of animals, is a very strong point in favor of their not being degeneration forms.

In general we may say the same things in regard to the relation between structure of the "bodies" and the variety, etc., of the animal, that we did when discussing size, because their structure varies to a certain extent with their size. The tiny forms, rounded, with a more or less centrally situated chromatin-staining granule, slightly larger forms with three to several such granules (often four), elongated forms with a central chromatin line, and tiny forms in twos or in groups of three or more (Plate 19, Figs. 3-8) are the only types found in fixed virus (with an occasional slightly larger form containing a larger central body and a few tiny granules). The tiny forms found in fixed virus seem to be far more delicate than apparently equally tiny forms seem in other lesions; that is, they take the stain more delicately, the central structure is not so distinct, and the whole body is more easily destroyed by pressure in the former than in the latter case. Hence, it is only in the best made smears that these fixed-virus forms are seen, and then only after the eye has been accustomed to their very delicate coloring and outline.

The forms found in fixed virus animals are the only ones which are better preserved or at least which are more distinctly seen in sections than in smears. This is due probably to their extreme delicacy. The fact that we have found very many forms in all cases (15) of developed fixed-virus infection studied makes it probable that they are present in every case and that they come out better with the technic

described in Part I. than with the technic followed by other investigators. In regard to their specificity, we would say that we have made few controls for the following reason. As slight alterations in technic seem to interfere with their demonstration, and as, therefore, their non-appearance might not mean that they are not present, large numbers of animals would have to be examined before one could be sure that forms simulating them might not be present in certain cases. The facts, however, that in our four controls and in the first two days after inoculation of a series of ten experimental rabbits (see below for details of this experiment), they are not found, and that when they do appear they possess certain characteristics, in structure, site, and number corresponding to the course of the disease, makes it pretty evident that we are dealing with the specific organism. These bodies have the following characteristics: They are tiny rounded forms, sometimes wavy in outline, as if possessing slight amoeboid motion, sometimes elongated, extending along the rim of the host-cell nucleus, or along one of the nerve fibrils, as if moving there; they take a delicate light magenta stain very similar to that taken by the small serum globules in the blood vessels, and it would be difficult, if not impossible, to distinguish some of them from these serum globules, if they were in the blood vessels. Many of the organisms, however, show a small chromatin granule, situated more or less eccentrically, sometimes on the very rim of the body. In the larger forms the granule is large; in the smaller it cannot always be seen (Plate 18, Fig 1, "Journal of Infectious Diseases," 1906). Some of the larger forms show from two to several granules and occasionally there is a body with the definite central body and the small granules about it. In these fixed-virus sections we have found certain tiny bodies in some of the nerve-cell nuclei, especially in the smaller of those cells which show decided degenerative changes of the cytoplasm. These intranuclear forms seem to stand out quite distinctly from the rounded, acid-staining degenerative masses. The latter are not so refractive as the former. The intranuclear forms have not yet been studied sufficiently to allow a decided opinion in regard to their place in the life-history of the organism. They are quite frequent in the olfactory bulbs of guinea pigs after inoculation with rabbit-fixed virus.

The fact that none of the larger forms of the "bodies" are found in animals dying after fixed-virus inoculations is an added indication that the bodies are not products of degeneration of the host cells.

That the development of only these tiny forms with their simple structure in fixed-virus animals is due to the fact that the special strain inoculated is accustomed to the one variety of host is shown by the result obtained by inoculating the strain into another variety of animal. We have inoculated one dog and several guinea pigs subdurally, and three mice subcutaneously with fixed virus from the rabbit, and in each case (in only one case in mice, as only one of the three died) besides the tiny forms there have been numerous large forms with the characteristic, definite, more or less complicated structure (corresponding to Plate 19, Figs. 17-34, "Journal of Infectious Diseases," 1906). This is contrary to the results obtained by Schiffmann upon inoculating rabbit-fixed virus into dogs. In his cases he could find no bodies whatever. On the other hand, we have had delayed fixed-virus action in one rabbit (inoculated with 2 c.c. of a thin emulsion into the ear vein, with death on the 11th day after typical symptoms of paralytic rabies), and in this animal we found only the tiny delicate forms found in the other fixed-virus rabbits.

In regard to variations in structure at different stages of the disease, most of our study has been made upon animals inoculated with fixed virus, and the forms and structure in these cases seem to be about the same in the early stages as in later ones. It would seem that under these favorable conditions for the organism, it grows and divides so rapidly from the beginning, and infects so many of the host cells, that the animal is overwhelmed before the parasite has a chance to develop the larger forms. The results are different in the animals inoculated with street virus.

We inoculated one series of seven rabbits with street virus from a dog, killed the first animal on the seventh day after, and the others respectively on the 9th, 11th, 12th, 14th, 16th, and 17th days. The results as to number and structure of the bodies are briefly as follows:

Seventh day Rabbit.—In the bodies of the large nerve cell of Ammon's horn and cerebral cortex an occasional tiny form and an occa-

sional one of the intermediate grades was seen. (Forms corresponding to Plate 19, Figs. 3-16, "Journal of Infectious Diseases," 1906.)

No definite extracellular forms were seen, but neither sections nor smears have yet been studied minutely. This is the earliest day reported for forms found after inoculations with street virus. Negri reports finding them on the 10th day in a dog. In our series of animals those that were allowed to remain alive did not begin to have visible symptoms until the 13th and 14th days.

Ninth-day Rabbit.—Many very definitely structured forms were seen in the large nerve cells of practically all parts of the cerebral nervous system, smears and sections showing equally well. The forms corresponding to Plate 19, Figs. 3-12 were in the majority, those corresponding to Figs. 13-16 in moderate numbers, and those corresponding to Figs. 17-32 occasionally.

Eleventh day Rabbit.—Practically no difference between it and 9th day one.

In the 12th, 14th, 16th, and 17th day rabbits the larger forms appeared in gradually larger numbers and many more division forms were seen.

So far most of the study in this series has been made on the earlier stages.

There are no marked differences in the "bodies" found in different parts of the central nervous system of one animal dead 25 days after inoculation into the sciatic nerve. The general histologic lesions are more intense in the cord and there is a larger number of the larger "bodies" there than usual, but the "bodies" in the brain are about the same in number and structure as in animals dying from subdural inoculations.

Appearance of "bodies" in hanging drop.—So far, we have done only enough work with the hanging drop to make us realize that it is an extremely difficult method of study and needs most careful control at each step. There is no doubt that certain forms of the organism can be recognized; but the nerve tissue elements change so quickly, assuming flagellated and delicately granular forms which simulate those of known organisms that the control must be at one's side before one realizes that the object studied is not a living organism.

Detailed Characteristics of Structure—In smears as well as in sections, the *cytoplasm* appears quite homogeneous; there is no evidence of a reticulum, or of a granular structure outside of the definite chromatoid granules. The smears, however, have brought out one important point in regard to the cytoplasm more clearly than the sections, and that is that it is more basophilic than acidophilic in staining qualities. With the Giemsa stain, as we have seen in Part I., it takes the methylene-blue stain more than the eosin-red, and even with the simple cosin methylene-blue stain the protoplasm appears as a deep magenta unless much decolorized.

One of the points, then, which has been brought up against the protozoan theory falls to the ground. The cytoplasm takes the stain as does that of many well-known protozoa—the malarial organism, for instance.

In studying the *central bodies* of these organisms, as they appear in the smears, one of the first things noticeable is that they are not surrounded by a clear space—that there is no sign of a vacuolar appearance in the whole body. This is a very different appearance from that given in the sections, and it shows that the vacuoles described in the sections are artefacts due to the technic. We notice next that in the great majority of the organisms the central body stands out clearly, as decidedly different in structure, and slightly so in staining qualities, from the chromatoid granules which surround it. The general type of the structure of the central body is that of well-known protozoan nuclei; for example, Prowazek gives a description of the nucleus in certain stages of the *Plasmodiophora brassicae*, which might be used here to describe the most typical appearance of these central bodies.

The chromatin is arranged in a more or less granular ring around the periphery of the central body or nucleus leaving an achromatic or more acid-staining center in which is situated, generally eccentrically, a varying-sized karyosome (Plate 19, Fig. 37, "Journal of Infectious Diseases," 1906). There are a number of variations from this principal type, according to stage of development. Often the whole nucleus answers to the description of the compound karyosome given by Calkins in his description of the protozoan nucleus. In the tiny "bodies" the chromatin can only be seen as a dot; in those a little larger

it may be a large solidly staining granule, or a ring or rod, the latter often hour-glass shaped. In forms large enough for the characteristic structure to be developed and to be clearly seen, the central body may show evidence of fragmentation (Plate 19, Figs. 18, 38, 51, etc., "Journal of Infectious Diseases," 1906). Just such evidence of fragmentation is shown in many protozoan nuclei preparatory to division. It is interesting that forms showing this phase, and, moreover, very similar in general appearance to some of the forms seen here, have been depicted by Doflein in the early stages of the life-cycle of *Glugea lophii*, a myxosporidium, parasitic in the ganglion cells of a fish (*Lophius piscatorius*).^{*} The staining of the nucleus will be considered with that of the chromatoid granules.

The chromatoid granules are most frequently arranged in a more or less complete circle about the nucleus. They are somewhat irregular in outline and size, being occasionally ring-shaped, sometimes elongated, often in twos, due probably to active changes of growth and division. They take generally a more mixed chromatin stain than the chromatin of the nucleus. This fact is brought out in the Giemsa-stained smears. Here the nuclear chromatin takes generally a definite azure tint, while the chromatoid granules are more of a blue, though sometimes they may appear more red. That the red in the central body and granules is not an eosin-red, is shown first by its peculiar magenta tint, and second by the fact that when partly decolorized by methyl alcohol, the red color disappears from these structures leaving them a dark blue, while the cytoplasm is a pale blue-pink and the red blood cells are a definite eosin-pink. If a dilute methyl alcohol is used, an interesting series of differentiations in color may be obtained. Such a more or less regular arrangement of chromatoid granules in the cytoplasm of Protozoa is of frequent occurrence (Calkins, Minchin). It is a marked feature, according to the observations of one of us, in certain stages of the *Plasmodiophora brassicae*. The further changes in the central bodies and granules will be considered under division forms.

^{*} In Doflein's later classification (1901) he names this species *Nosema lophii* and places it in the sub-order Microsporidia under the order Cnidosporidia.

Different Shapes.—We agree with Negri in considering many of the different shapes due to the position of the organism in the host cell. There is no doubt that the substance of these bodies is extremely delicate and plastic, easily adapting itself to the position in which it is found and easily destroyed by artificial means. Many of the elongated forms are forms growing and dividing in this way because of position between the fibrils. The triangular forms (Plate 19, Figs. 26, 50, and Plate 21, Fig. 7, "Journal of Infectious Diseases," 1906) are probably forms that have grown in the angle made by the giving off of a nerve cell branch. They have been placed by us, in Plate 19, underneath the much elongated forms as possible division forms of the latter; but they probably are not. The principal cause of most of the different shapes, however, is the rapid growth and division of the organism.

Division Forms.—The whole picture is one of rapid growth and multiplication, and this corresponds with the clinical history. The elongated forms containing from two to five or even six nuclei are the result of rapid nuclear division without corresponding cell division. This condition is found quite frequently in Protozoa ("*Thelohania mulleri*," Minchin, p. 292). The elongation in this way is probably due, as we have said, to the position of these bodies between the nerve fibrils, and to their great plasticity.

Under the most favorable conditions (fixed virus), growth and division occur most rapidly and simply, the tiny forms dividing and redividing apparently indefinitely. Whether there is simple conjugation, or fusion of unequally divided forms during this condition, it is difficult to say. It would probably take much study to settle this question. Small mulberry masses are found during this stage, but whether they are the result of the breaking up of a larger form or of the rapid division of a tiny form it is impossible for us to say as yet. We have also seen appearances which suggest plasmodial phases. There seems to be distinct evidence of an intranuclear invasion also in fixed-virus infection.

In cases where there has been an inoculation of comparatively small quantities of the virus, i. e., a small number of forms of the parasite capable of immediate infection, or in cases where there has been an infection of less susceptible animals (dogs, cattle, human beings, etc.),

or with a less accustomed virus (fixed virus or rabbits into guinea pigs or mice), we get a slower growth, with its larger structures and different division forms. The chromatin accumulation in the form of a definite nucleus, apparently undergoes fragmentation very easily, and so we have forms containing two to several central bodies, some rounded (Plate 19, Figs. 12, 13, 14, 19, etc., "Journal of Infectious Diseases," 1906), some elongated (Fig. 15), some of unequal division, similar to budding (Fig. 29). Then we find forms with bodies apparently differentiated within one membrane (Figs. 20, 31, 53), and bodies with practically all stages of hour-glass constriction, indicating transverse division (Fig. 32). Many pairs, unequal in size, apparently fusing or dividing have been seen (Figs. 33, 45), and finally, we have large bodies with the chromatin scattered throughout the whole organism in the form of tiny, unevenly rounded or elongated masses, one or two larger, indicating the remains of the nucleus, and in these forms we get all stages of apparent budding (Figs. 40, 41, 42, 54, 55). The buds vary somewhat in size, some being very tiny. The formation of buds accounts for the appearance in the same cell of both large and small forms. It also helps to account for the rapid spread of the organisms. These tiny budded forms similar to "swarm spores" are probably motile and pass quickly to other host cells.

We have also found a number of more or less indefinite masses, taking the stain a little more deeply than the other bodies, and apparently made up of large numbers of tiny bodies, but so far they have been too indefinite for us to be sure that we have cystlike structures. We have not studied the sections minutely enough yet to find out how such structures appear there, or whether they are similar to the "cysts" described by Negri.

Conjugation Forms.—At first sight "the buds" were thought by us to be possibly conjugating individuals, but when on further study they were found to be principally, if not entirely, in forms which showed marked fragmentation of the chromatin, they were interpreted as budding forms. Such unequal forms as are represented in Plate 19, Figs. 33 and 45, may be conjugating forms, but so far we have not been able to decide as to their significance.

The relation between the time the central nervous tissue becomes infective, and the time the bodies appear.—Our principal work on this point has been done with fixed virus. After finding that tiny, characteristic forms were found in two rabbits dying on the eighth and ninth days after subdural inoculation with fixed-virus, we inoculated 10 rabbits subdurally with fixed virus (629th passage), killed one every day by chloroform, and examined the central nervous system in the following way: One-half of the brain and medulla, including the olfactory bulb, was cut into slices, and with slices from the dorsal and lumbar spinal cord, including one or two spinal ganglia, was placed in Zenker, and subjected to the technic for sections mentioned in Part I. From the other half of the brain, and corresponding parts of the cord, two sets of smears were made, and each stained respectively by the two methods mentioned in Part I. Unfortunately, with this series of animals, we did not test the virulence of the nerve tissues, so we do not know at exactly what period it became distinctly virulent. However, in an earlier series of eight rabbits inoculated in the same way, and from which only smears were made, Dr. Poor tested the virulence roughly, as follows: One animal was killed each day, with the exception of the eighth, which died on the ninth day. From the lumbar cord, and from Ammon's horn, pieces of about the same size, so far as we could judge from eye measurement, were cut. Two dilutions were made from each piece, a stronger one, by the addition of 3 c.c. of normal salt solution, making an emulsion; and a weaker one, by making a 1:1,000 dilution of the stronger. Two guinea pigs were inoculated with the weak dilution $\frac{1}{2}$ c.c. each; two with the strong dilution, $\frac{1}{2}$ c.c. each.

Of the animals inoculated with the weak dilutions of the *cord*, none died; of those inoculated with weak dilutions of the *brain*, none died from the first or second day rabbits, one died from the third day, and one from the fourth day animal, none from the fifth day, one from the sixth day, two from the seventh day, and none from the ninth day animal. Of the animals inoculated with the strong dilutions of the *cord*, none died from the first, second, third, and fourth day rabbits; one from the fifth day, one from the sixth day, and none from the seventh day animal. Eighth and ninth day animals were not inoculated. Of

the animals inoculated with the strong dilutions of the *brain*, none died from first and second day rabbits, two died from the third, fourth, fifth, sixth, and seventh day rabbits, eighth and ninth day animals not inoculated.

In this experiment, then, the weak dilution of the *cord* was not infective in the doses used; the strong dilution was not infective until the fifth day, and then not regularly so; while both dilutions of the *brain* became infective on the third day, the weaker one less so, and continued so to the end. These results corroborate the work of Remlinger, who found the medulla virulent on the third or fourth day after subdural inoculations of fixed virus.

In neither of these sets of experiments has the approximate number of organisms present been shown, and until we know this we cannot say that in any measured amount of infective material there may be more than an occasional tiny form, which it might be very difficult, perhaps impossible, to find in sections or smears of such material.

In the examination of the 10 rabbits mentioned first in this connection, although we have so far studied only a comparatively few sections, we have found the bodies appearing as follows: On the first and second days none; on the third day an occasional one in the large lymphoid cells of the perivascular lymph spaces at the base of Ammon's horn; on the fourth day, a few tiny undoubted ones in the large nerve cells of the olfactory bulb, of the lower curve of Ammon's horn, and of the motor area of the cerebral cortex; on the fifth, a moderate number in the same areas and in scattered cells throughout the whole brain; on the sixth, many in the same areas, and in the medulla; on the seventh two animals), on the eighth, and on the ninth, very many, as in the other fixed-virus animals studied (Plate 18, Fig. 1, "Journal of Infectious Diseases," 1906).

From this series of experiments it seems that the bodies may be found soon enough and in practically large enough numbers to account for the beginning infectivity of the nerve tissue, and that with only a little more careful experimenting this may be brought out clearly.

Four control rabbits were studied in this connection; two normal rabbits, one which had died from pneumococcus infection, and one from yeast infection.

Spread of the bodies to different parts of the host.—This point is now being studied by us. It is taken up under two heads; first, the spread of the organisms from the point of inoculation, and second, its spread from the site of infection.

In whatever way the virus enters the body, so far as we know, there is no development of the organism, or none, to any appreciable extent, until it reaches the central nervous system, and not until after a certain amount of development there does it infect the peripheral organs. Before the disease was well studied it was thought that the salivary glands were the chief site of the infection. But it has been shown that these glands are not always infective, and when they are, not until comparatively late in the disease and that when the virus is inoculated into them, the animal seldom comes down with the disease and probably never if the centripetal nerves are cut (Bertarelli). This means that the parasite does not grow in the salivary glands, that it is only carried there incidentally by its spread from the central nervous system along the nerve branches. That the organisms escape into the blood and are carried in this way in small numbers is shown by the fact that the blood in large quantities has been found infective (Marie). Principally by the nerve channels, secondarily by the blood and lymph channels, the organisms are carried in small numbers to all parts of the body. With other investigators, we have found the suprarenal capsules infective (in one out of two street-rabies dogs). One of the three guinea pigs inoculated died after typical symptoms of rabies, and the central nervous system showed many good-sized bodies and was infective for other animals. If it is true that the organisms pass in such comparatively small numbers to the various peripheral organs, and especially if only the smaller forms pass, then our chances of identifying them in the salivary and other glands are very slight. Smears from these parts are unsatisfactory, and we have not yet been able to study the sections.

In regard to the spread of the organisms from the point of inoculation, the parasites are probably carried to the central nervous system along channels similar to those by which they are carried away, and unless enough of them can quickly reach the nerve cells, they are probably destroyed by the macrophages. We have found, as we have

said, what appear to be tiny bodies in the large lymph cells on the third day after inoculation with fixed virus. In one fixed-virus rabbit, found dead on the morning of the seventh day after inoculation, an animal which had been used before, and whose resistance was probably lessened, the central nervous system was loaded with large lymphoid cells many of which were apparently filled with tiny organisms. This question is still being studied.

Significance of the "bodies" and comparison with known organisms.—Although it may be questioned whether enough forms have been found to account for every stage in a life-cycle, it is certain that the great majority of the bodies stand out so clearly as organisms with such definite, constant, characteristic structure and staining reactions and show so many forms similar to division forms of known Protozoa, that the picture is difficult to explain in any other way than as that of a developing organism belonging to the group Protozoa. It seems unnecessary further to consider the possibility of their being changed red blood cells or any other form of degeneration of the host tissue; and this alone is evidence in favor of their being organisms.

From time to time cases have occurred in which the "bodies" are seen in such numbers and in such stages of development that we are as sure of their being organisms as we are that the bodies photographed by Wright from Delhi boil, are organisms. As we study the picture further and find at almost every step analogies in the life-cycle of known Protozoa, the evidence is so overwhelming that there seems no reason to doubt that they are living organisms; the small single forms with their tiny chromatin central bodies rounded, elongated, or in twos and more, as in *Nosema lophii* and other Microsporidia (Doflein); the groups of small forms in twos and more (multiplicative reproduction of Doflein); the appearances of the central body in the larger forms similar to that of many protozoan nuclei at corresponding stages of development (Calkins, Prowazek); the many evidences of division of these larger forms such as fragmentation of the nucleus (Calkins), two nuclei, all stages of hour-glass constriction of the body; and finally, the distribution of the nuclear material throughout the whole organism with evidences of its fragmentation and of budding, a phenomenon which has been described as occurring in all classes of

Protozoa (Calkins, Minchin)—all these and more make a collection of evidence which amounts to proof.

The parasite seems to possess more points of resemblance to organisms belonging to the sub-order Microsporidia, than to those of any other order.

SUMMARY AND CONCLUSIONS.

1. The smear method of examining the Negri bodies is superior to any other method so far published for the following reasons: (*a*) It is simpler, shorter, and less expensive; (*b*) The Negri bodies appear much more distinct and characteristic. For this reason and the preceding one, its value in diagnostic work is great; (*c*) The minute structure of the Negri bodies can be demonstrated more clearly; (*d*) Characteristic staining reactions are brought out.

2. The Negri bodies as shown by the smears as well as by the sections are specific to hydrophobia.

3. Numerous "bodies" are found in fixed virus.

4. "Bodies" are found before the beginning of visible symptoms—i. e., on the fourth day in fixed virus, on the seventh day in street virus, and evidence is given that they may be found early enough to account for the appearance of infectivity in the host tissues.

5. Forms similar in structure and staining qualities to the others, but just within the limits of visible structure at (1,500 diam. magnification) have been seen. Such tiny forms, considering the evidence they give of plasticity, might be able to pass the coarser Berkefeld filters.

6. The Negri bodies are organisms belonging to the class Protozoa. The reasons for this conclusion are: (*a*) They have a definite, characteristic morphology; (*b*) This morphology is constantly cyclic, i. e., certain forms always predominate in certain stages of the disease, and a definite series of forms indicating growth and multiplication can be demonstrated; (*c*) The structure and staining qualities as shown especially by the smear method of examination resemble that of certain known Protozoa, notably of those belonging to the sub-order Microsporidia.

7. The proof that the "Negri bodies" are living organisms is sufficient proof that they are the cause of hydrophobia; a single variety

of living organisms found in such large numbers in every case of a disease, and only in that disease, appearing at the time the host tissue becomes infective in regions that are infective, and increasing in these infective areas with the course of the disease can be no other, according to our present views, than the cause of that disease.

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THE ELECTRICAL CHARGE OF TOXIN AND ANTITOXIN.

By CYRUS W. FIELD and OSCAR TEAGUE.

Soon after the discovery of diphtheria antitoxin, several investigators attempted to convert diphtheria toxin into antitoxin by the electrical current, and some went so far as to suppose that this method would supersede the costly and time-consuming process of immunizing animals. Smirnow^a inoculated rabbits with half a cubic centimeter of a two to three days old broth culture of diphtheria bacilli, and twenty-four hours later, when the animals were sick, injected 10 cubic centimeters of the anodal fluid, obtained by passing a current for eighteen hours through diphtheria toxin. According to him, the animals were saved by the injections. Bolton and Pease^b stated that two cubic centimeters of the anodal fluid obtained from diphtheria toxin neutralized ten minimal lethal doses of the toxin. It is a well-established fact that acids destroy diphtheria toxin more readily than alkalis, and hence it is to be considered that it was the acid at anode which in Bolton and Pease's experiments neutralized the toxin. The latter investigators believed that the electric current caused a rearrangement of the constituent atoms of the toxin molecule, so that antitoxin resulted; but they did not determine whether the toxin molecule moved with or against the current by virtue of the charge which it carried.

The first to undertake the determination of the electro-positive or electro-negative nature of diphtheria toxin and antitoxin was Römer^c. Römer used a U-shaped tube and allowed the electrodes to dip into the toxin and antitoxin to be investigated. After the current had been passed for a stated interval of time, the fluid was pipetted from both branches of the tube simultaneously and tested on guinea pigs for toxic or antitoxic properties. The results were entirely negative, since he was unable to determine whether the toxin or antitoxin particles traveled toward the cathode or anode. This failure was due primarily, we believe, to the destruction of the toxin and antitoxin by the products of electrolysis, brought about by the strong current employed, for

^a Smirnow, *Berl. klin. Woch.*, 1892, xxxii, 645.

^b Bolton and Pease, *Jour. of Exper. Med.*, 1896, i, 537.

^c Römer, *Berl. klin. Woch.*, 1904, xli, 209.

Römer states that he used oil to get rid of the bubbles at the electrodes. Furthermore, the method of simultaneous pipetting very likely caused some mixing of the different portions of the fluid.

To avoid these errors in technique and to eliminate the effects of electrolytic products, we first used a modification of Bilitzer's cells, as shown in Figure 1. The cells and connecting tubes were of glass;

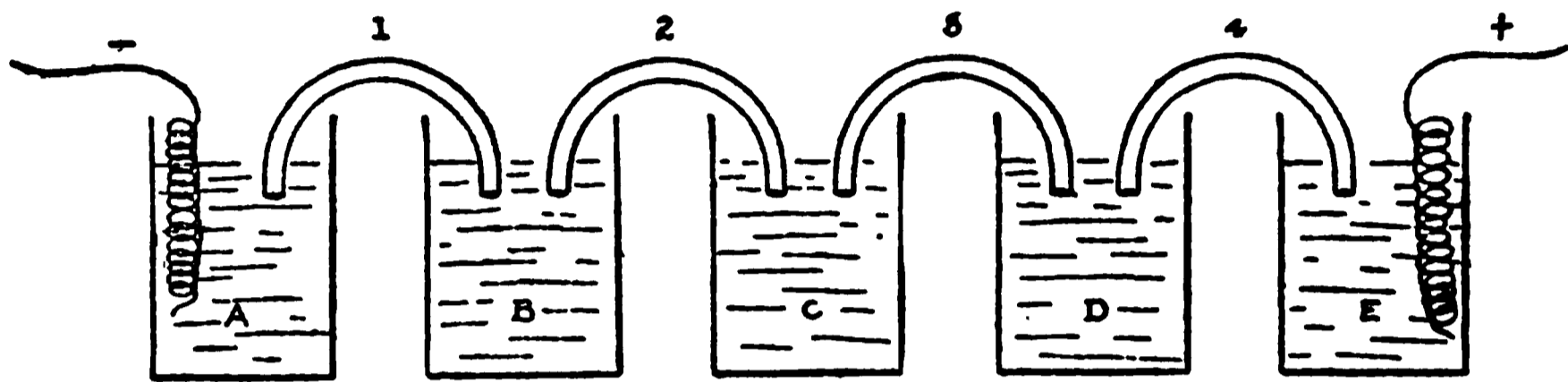


FIG. 1.

the electrodes consisted of coils of platinum wire. The cells *A*, *B*, *D* and *E* were filled with distilled water, which was brought to the same level in all of them by means of water-filled connecting tubes. The central cell, *C*, was then filled to a slightly lower level than the others with the toxin or antitoxin to be tested. The small connecting tubes 1 and 4 being in place, and the direct lighting current turned on, the tubes 2 and 3 were filled with distilled water and simultaneously placed in position, thus completing the circuit. After the current had been passed for the desired length of time, the connecting tubes 2 and 3 were removed at the same moment, care being taken that they remained filled. Then 1 and 4 were removed. Under the influence of the current, the fluid in *A* became alkaline, that in *E*, acid, while *B* and *D* remained almost neutral. Hence, only the fluid in the two latter cells was tested on guinea pigs for toxic or antitoxic value. The results were unsatisfactory and contradictory, probably owing to the fact that some of the test fluid passed into the neighboring cells along the outside surface of the connecting tubes, being drawn up by capillarity. When we used longer connecting tubes, the internal resistance became too great for the passage of an appreciable amount of current. This method was therefore discarded.

The following apparatus yielded decisive results. Two semi-circular glass tubes, each 1 cm. in diameter and 20 cm. long, were filled

with melted agar (2 per cent. agar in distilled water) and allowed to cool to the temperature of the room. These were then arranged as in Figure 2, the same platinum electrodes being used as in the previous experiments. The toxin or antitoxin to be tested was placed in the middle beaker, distilled water in the other two. The direct Edison

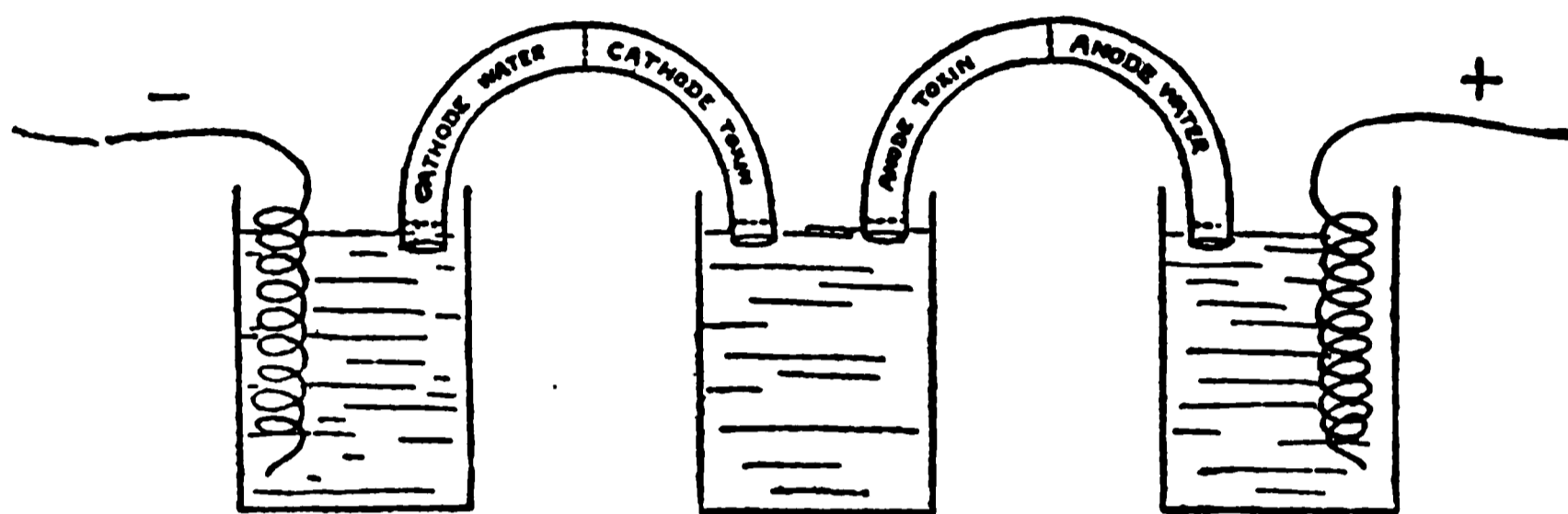


FIG. 2.

street lighting current, 110 volts, was passed for from four to five hours^d, the distilled water in the end beakers being siphoned off and renewed every half hour to eliminate the disturbing influence of the products of electrolysis. The agar tubes were now removed and the ends thoroughly rinsed in distilled water. The agar mass was then forced from that portion of the tube which dipped into the toxin or antitoxin out through the other end. About a quarter of an inch was removed from each end of the mass and discarded; the remainder was divided approximately in half. Each of these portions was rinsed in distilled water, chopped into fine pieces, and allowed to stand for one hour in about 6 c.c. of distilled water. At the end of this time the agar was removed by filtering through gauze, and the filtrate was tested on guinea pigs for toxic or antitoxic properties. The accompanying table gives the results of these experiments.

^d The current was passed for only four or five hours because the risk of a disturbance due to products of electrolysis increased with the time; moreover, Bredig, Hardy, Pauli, and others found that the passage of a current for twenty-four hours or more would often cause a reversal of the charge carried by particles which would then necessarily be driven back in the opposite direction, thus obscuring the nature of the charge which they originally carried.

TABLE I.

Substance Tested by Passage of Electric Current.	Acidity or Alkalinity.	Cathode Water.	Cathode Toxin or Antitoxin.	Anode Toxin or Antitoxin.	Anode Water.
Diphtheria toxin	{ Acid..... Alkaline...	No reaction. No reaction.	† In 20 hours. † In 28 hours.	No reaction. No reaction.	No reaction. No reaction.
Tetanus toxin.....	{ Acid..... Alkaline...	No reaction. No reaction.	† In 48 hours. † In 40 hours.	No reaction. No reaction.	No reaction. No reaction.
Diphtheria antitoxic serum. Tested against 35 M.L.D.'s.	{ Acid..... Alkaline...	Protected. Protected.	Protected. Protected.	† In 22 hours. † In 42 hours.	† In 24 hours. † In 38 hours.
Diphtheria antitoxic globulins. Tested against 35 M.L.D.'s.	{ Acid..... Alkaline...	† In 36 hours. † In 24 hours.	Protected. Protected.	† In 36 hours. † In 36 hours.	† In 36 hours. † In 36 hours.
Tetanus antitoxic serum. Tested against 35 M.L.D.'s.	{ Acid..... Alkaline...	Protected. Protected.	Protected. Protected.	† In 50 hours. † In 48 hours.	† In 68 hours. † In 48 hours.
Normal broth. No toxin	{ Acid..... Alkaline...	No reaction. No reaction.	No reaction. No reaction.	No reaction. No reaction.	No reaction. No reaction.
Normal Horse serum. No antitoxin. Test- ed against 35 M. L. D.'s.....	{ Acid..... Alkaline...	† In 36 hours. † In 40 hours.	† In 36 hours. † In 42 hours.	† In 36 hours. † In 40 hours.	† In 36 hours. † In 40 hours.
Test without the electric current			Water-half negative, † in 40 hours.		Toxin-half negative, † in 41 hours.
Diphtheria toxin					
Diphtheria antitoxin. Tested against 35 M. L. D.'s.....					

The first .5 cm. of the agar dipping into the toxin or antitoxin was removed, as was always done in our experiments with the electric current. There was always a slight trace of toxin or antitoxin in the first half centimeter, but it was never found beyond this point. A similar phenomenon was observed by Flexner and Noguchi, ^f in relation to the diffusion of tetanus toxin into agar.

In order that the table may be more readily understood, a detailed explanation of one of the experiments (No. 6, for example) contained in it may not be superfluous.

Diphtheria antitoxic serum was made slightly alkaline to phenolphthalein by the addition of a small amount of 0.1 normal sodium hydrate. The agar tubes were placed in position, as in Figure 2, and the current was passed through it for four hours. The four portions of agar, which we shall designate as cathode water, cathode-antitoxin, anode water, and anode-antitoxin, were then cut into fine pieces and extracted in water for one hour, and to each of these ex-

^c Gibson, *Jour. of Biol. Chem.*, 1905, i, 161.

^f Flexner and Noguchi, *Jour. of Exper. Med.*, 1906, viii, 547.

tracts was added 35 m. l. d. of diphtheria toxin. These four portions of fluid were now injected subcutaneously into the abdominal walls of four guinea pigs each weighing about 250 grams.

The two guinea pigs receiving the fluid from the anode water and anode-antitoxin portions of agar died in thirty-eight and forty-two hours, respectively; hence, these portions must have contained no antitoxin. Those receiving the fluid from the cathode water and cathode-antitoxin portions showed no induration at the point of injection and no loss of weight for five days, after which they were discharged. Hence, they were fully protected against the 35 m. l. d. of toxin.

The table shows that under the influence of an electric current, the particles of both toxin and antitoxin travel toward the cathode, and that a change in the reaction of the solvent does not cause a reversal of the charge carried by the particles.

Biltz, Much and Siebert^g claim that both tetanus toxin and tetanus antitoxin are precipitated by electro-positive inorganic colloids and conclude, therefore, that they are electro-negative and should, under the influence of an electric current, pass toward the anode. However, neither they nor Römer were able to demonstrate this fact experimentally. It is probable that the precipitates which they obtained were due either to the action of electrolytes contained in the toxin or antitoxin on their inorganic colloids, or to the action of these colloids on non-toxic or non-antitoxic protein substances, or to a combination both. It is worthy of note that their non-toxic broth gave precipitates with practically the same inorganic colloids as did the toxin.

Hardy^h states that proteins are amphoteric; that is, that in an acid medium they travel toward the cathode and in an alkaline medium toward the anode, while in a neutral medium they do not move toward either pole. He worked with an albumen coagulated by heat. Pauliⁱ, using a protein solution obtained by dialyzing serum from eight to ten weeks and filtering off the euglobulin, found, in agreement with Hardy, that the protein was electro-positive in an acid solution and electro-

^g Biltz, Much and Siebert, *Beit. zur exper. Therapie*, 1905.

^h Hardy, *Jour. of Physiology*, 1899, xxiv, 288.

ⁱ Pauli, *Hofmeister's Beit.*, 1906, vii, 531.

negative in an alkaline one^j. We have shown that alteration of the reaction of the solvent does not change the character of the charge carried by particles of toxin or antitoxin. If proteins are amphoteric, as is generally believed, then this observation of ours would point to the non-protein nature of toxin and antitoxin. In one series of experiments, however, we have found that the portion of agar containing the toxin or antitoxin, that is, the cathode portion, was the only one to give the biuret reaction and this occurred when the test substance was in either an acid or an alkaline solution. If experiments which we have under way should verify the last results, it would indicate that native proteins may not be amphoteric and then the argument that we have advanced as to the non-protein nature of toxin and antitoxin would be invalidated^k.

If the combination of toxin with antitoxin is a true chemical reaction, one would expect that under the influence of an electric current toxin would travel in one direction and antitoxin in the opposite direction. Such, however, was not found to be the case and we are, therefore, inclined to believe that this union is not a true chemical reaction, but a matter of adsorption, as was first suggested by Bordet, and has since been claimed by others.

Conclusions.

1. Both diphtheria and tetanus toxin and their antitoxins are electro-positive, that is, they pass to the cathode under the influence of an electric current.
2. The character of the charge is not altered by a change in the reaction of the solvent.
3. The combination of toxin and antitoxin would seem to represent not a true chemical reaction but the adsorption of one colloid by another.

^j Pauli denaturalized his proteids by the prolonged dialysis.

^k Oppenheimer (Toxin und Antitoxin, 1903) in summing up the work on this subject concluded that toxin and antitoxins are of non-protein nature. Quite recently Osborn, Mendel and Harris (*Amer. Jour Physiol.*, 1905, xiv, 259), working with ricin, have taken issue with him, having found that their purest product still gave protein reactions. As a matter of fact, nothing definite is known at present of the chemical nature of these various substances.

THE ELECTRICAL CHARGE OF THE NATIVE PROTEINS AND THE AGGLUTININS.

By CYRUS W. FIELD and OSCAR TEAGUE ^a.

In a previous paper ^b it was shown that the particles of both toxin and antitoxin wandered under the influence of an electric current toward the cathode and that the reaction (acidity or alkalinity) of the solvent did not influence the direction of migration. Since Hardy ^c and Pauli ^d demonstrated that the proteins which they used were amphoteric, *i. e.*, that they pass toward the anode in an alkaline medium and to the cathode in an acid one, there has been a tendency to generalize by assuming that all proteins behave in this manner. If such were the case, we pointed out, it would follow from our experiments that toxin and antitoxin are not true proteins. At the same time, however, we mentioned that from the few experiments in which this question had been considered, the protein matter of the broth or serum seemed in every instance to travel with the toxin and antitoxin toward the cathode. Further experiments have confirmed this result. It was also shown that the protein of normal horse serum and of non-toxic broth travels toward the cathode. Hence our work offers as yet no evidence either for or against the view that toxin and antitoxin are non-protein in nature.

We maintain that the results which Hardy and Pauli obtained, working with denaturalized proteins, are in nowise applicable to the native proteins, but that the latter carry a distinct electrical charge and are not amphoteric. We are here in accord with Iscovesco ^e and his co-workers, who investigated the charge of colloids contained in various body fluids. Their method consisted in treating the fluid with electro-negative (arsenic sulphide) and electro-positive (ferric hydrate) inorganic colloids and their conclusions were based upon the fact that colloids of opposite sign when brought together form precipitates. Thus they found that the peritoneal fluid of the horse contains only electro-

^a Assisted by a grant from the Rockefeller Institute for Medical Research.

^b Field and Teague, *Journal of Exper. Med.*, 1907, viii; also p. 50, this volume.

^c *Jour. of Physiol.*, 1899, xxiv, 288.

^d *Hofmeister's Beit.*, 1906, vii, 531.

^e *Compt. rend. Soc. Biol.*, 1906, lxi, 195, 355, 378, 470, 568.

positive colloids, while the pericardial fluid contains those of both signs; that blood plasma contains both positive and negative albumins with positive and negative globulins, whereas the serum contains only the positive globulin along with albumins of both signs; that the fluid of a tubercular abscess deprived of its leucocytes contains only electro-negative colloids; that the amniotic fluid contains both positive and negative albumins, but only negative globulins. From these experiments Iscovesco concludes that there are no colloids which do not bear a distinct electrostatic charge.

Since our method gave no indication of the presence of an electro-negative albumin in normal serum, we are inclined to believe that Iscovesco by his manipulations produced a change in sign of the charge carried by certain proteins and that all of his findings are therefore not applicable to the proteins originally present in the fluids he investigated.

In our previous work with tetanus toxin we investigated only the tetanospasmin and its antibody; we have since shown, by testing the agar extracts for their lytic or antilytic action on horse cells, that both tetanolysin and antitetanolysin travel toward the cathode under the influence of an electric current. Having determined the electrical charge of toxin and antitoxin, we next applied the same method to an investigation of the agglutinins.

The agar was divided into one centimeter lengths; the agglutinin was found to have traveled seven centimeters into the cathode agar, the anode agar remaining free of agglutinin. The first centimeter length was extracted with five cubic centimeters of water and this extract would still agglutinate at a dilution of 1-100.

The specific agglutinins investigated travel toward the cathode. These results are diametrically opposed to those of Biltz, Much and Siebert^f, who are the only workers, so far as we know, who have investigated this subject. They passed a current through lacto-serum contained in a U-shaped tube for from one-half to one hour, and found that the fluid around the anode agglutinated at 1-20, that around the cathode not at all, and that from the middle of the U-shaped tube at

^f *Zeit. für diätet. und physikal. Ther.*, 1905, viii. 19.

1-8. Normally the serum agglutinated at 1-4. They state that after the passage of the current the fluid from around the anode was 1-10 normal acid. We would expect this amount of acid to agglutinate at approximately 1-20, since 1-200 represents about the flocking limit of hydrochloric acid for bacteria.

As stated in a previous article, we took special precautions to eliminate the products of electrolysis. However, to show conclusively that it was the specific agglutinin, and that alone, which was responsible for the agglutination in our experiments, the extracts were also tested against other bacilli than those which were agglutinated by the serum under investigation.

TABLE I.

Strength of Electric Current 110 Volts; ½ to 1 Milli-ampere.

Serum agglutinating the typhoid bacillus at 1/2000. Current passed for six hours.

Organism.	Cathode Agar cm Lengths.							Anode Agar.		
	1	2	3	4	5	6	7	20	1-10	11-20
<i>B. typhosus</i>	+++	+++	+++	+++	++	++	+	o	o	o
<i>B. coli</i> ..	o	o	o	o	o	o	o	o	o	o
Shiga's bacillus.....	o	o	o	o	o	o	o	o	o	o
Paratyphoid b.....	o	o	o	o	o	o	o	o	o	o
Biuret reaction	+	+	+	+	+	+	trace			

NOTE—As one centimeter lengths of the anode agar showed no agglutinin in repeated experiments we have here tested extracts from ten centimeter lengths.

If the agglutination were due to the presence of products of electrolysis we would expect the other bacilli to be agglutinated as well as typhoid. Such, however, was not the case. Hence, we believe that we have shown conclusively that the agglutinins travel toward the cathode.

It has been shown by Bechtold^g, and by Buxton, Schaeffer, & Teague^h, and by others, that bacteria move toward the anode under the influence of an electric current, that is, they carry a negative chargeⁱ. Our findings with regard to the agglutinins are therefore

^g *Zeit. Physik Chem.*, 1904, xlviii, 385.

^h *Ibid.*, 1906, lvii, 47.

ⁱ Cernovodeanu and Henri (*Compt rend. Soc. de Biol.*, 1906, lxi, 200) claim that dysentery bacilli travel toward the cathode but we have not found this to be the case.

especially interesting, since it shows that in the phenomenon of agglutination we have the combination of an electro-negative suspension with an electro-positive colloidal solution. Since ions of opposite sign are essential for a chemical reaction, and colloids of opposite sign when brought together form precipitates, our results harmonize with both the chemical and the colloidal view of the phenomenon.

Bacteria which have been saturated with agglutinin and then washed in a number of changes of water until the wash water contains no more agglutinin were placed in the cell, and after eight hours the agar was tested for agglutinin. A small amount was found in the cathode agar showing that under the influence of the electric current the agglutinin-bacteria combination was dissociated and that the agglutinins passed to the cathode. Bacteria have been dissociated from agglutinins by other means^j, but so far as we are aware, this is the first time that dissociation has been effected by means of the electric current.

Conclusions.

1. Tetanolysin and antitetanolysin travel toward the cathode under the influence of an electric current.
2. The specific agglutinins are electro-positive.
3. The proteid matter of serum is not amphoteric but travels toward the cathode whether its reaction be acid, neutral, or alkaline.
4. The bacteria-agglutinin combination may be dissociated by means of the electric current.

^j Quoted by Eisenberg in *Cent. f. Bakt*, 1906, xxxi, 540, are the following: Joos (if fresh bacilli are added to agglutinated bacilli, which had been previously washed free from serum, the former are agglutinated), Landsteiner and Jagic (dissociation at high temperatures) and Landsteiner and Reich.

EXPERIMENTS ON THE PRODUCTION OF ANTIRABIC SERUM.

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Bacteriologist,

Assisted by PHILIP J. FRIEDMAN, B. S.,
Laboratory Assistant.

The objects of this work were to determine, (1) if it was possible to produce a serum cytolytic or cytotoxic against the rabies organism, and (2) if such a serum could be combined with the Pasteur treatment to hasten the production of immunity in severe head bites, a class of cases in which the Pasteur treatment alone occasionally fails on account of the shortness of the incubation.

This combination of vaccine and serum has been used successfully in rinderpest and anthrax.

Most of the work on antirabic serum has been done by A. Marie of the Pasteur Institute in Paris. In 1902 he stated: "We know that the serum of mammals vaccinated against rabies possesses the power of neutralizing the rabic virus 'in vitro.'" Marie further states that for the production of such a serum the animal must be injected with strong virus during a long period of time.

In 1904 Victor Babes reported a series of patients severely bitten by rabid wolves in which he gave a combination of the Pasteur treatment and the serum of an immunized dog. The number of cases thus treated was not sufficiently large to judge of the advisability of adding the serum to the treatment.

Our work along this line may be tabulated as follows:

1. Animals used for the production of the serum. The dog, sheep, rabbit and horse, all of these have produced a strong immune serum; no others have been tried.

2. The treatment of the animal supplying the serum. The animals have been treated for variable lengths of time, usually a number of months. The treatment of a horse which produced a strong serum in a short time is appended:

Date.	Dose.	Virus.
September 19.....	40 c.c.	{ Filtered emulsion of brain of fixed virus rabbit heated to 65 degrees for 20 minutes.
September 20.....	40 c.c.	{ Filtered emulsion of brain of fixed virus rabbit heated to 65 degrees for 20 minutes.
September 24.....	60 c.c.	{ Filtered emulsion of brain of fixed virus rabbit heated to 65 degrees for 20 minutes.
September 25.....	60 c.c.	{ Filtered emulsion of brain of fixed virus rabbit heated to 65 degrees for 20 minutes.*
September 26.....	30 c.c.	6-day dried cord.
September 28.....	30 c.c.	4-day dried cord.
September 29.....	30 c.c.	3-day dried cord.
September 30.....	30 c.c.	2-day dried cord.
October 1.....	30 c.c.	4-day dried cord.
October 2.....	30 c.c.	3-day dried cord.
October 3.....	30 c.c.	2-day dried cord.
October 9.....	30 c.c.	3-day dried cord.
October 10.....	30 c.c.	1-day dried cord.
October 11.....	30 c.c.	2-day dried cord.
October 15.....	30 c.c.	2 and 3-day dried cord.
October 17.....	30 c.c.	Fresh cord.
October 18.....	30 c.c.	2-day dried cord.
October 22.....	10 c.c.	Fresh brain.
October 28.....	30 c.c.	Fresh brain.

* These emulsions were made in the proportion of 1/5 of an inch of cord to 3 c.c. of physiological salt solution.

It may be said that two sheep are at present under treatment which receive the relatively large doses of 20 c.c. of fresh brain emulsion without apparent detriment.

November 12, 2 litres of blood drawn from the jugular and tested November 13.

3. Technique of the treatment of the serum in vitro. Where it is desired to compare the strength of different animal sera or that of the same sera at different times, it is evident that a virus of uniform strength is desirable. This virus is prepared by us in the following way: A rabbit is autopsied on the eighth day after subdural inoculation with fixed virus. One gram of the right hemisphere (taken from before backward) is emulsified with 12 c.c. of normal salt solution. This emulsion is then centrifuged for three minutes under fixed conditions of rate of speed, tube, etc. A fixed amount of the top layer of the

supernatant fluid is then drawn off and used as the test virus. This gives a virus sufficiently strong to kill a guinea pig in 5-6 days and sufficiently dilute to be free from particles of brain tissues of sufficient size to interfere with the action of the serum. It seems to me that such a virus would be of about as constant a strength as it is practicable to use.

This virus is mixed with the serum to be tested in various proportions and allowed to stand at room temperature 30 to 45 minutes. At the end of this time equal doses of these mixtures and of a control virus are inoculated into guinea pigs subdurally and at the end of a week the test is complete.

The following test which was made on the horse serum referred to above, serves as an example. On November 15 the following mixtures were inoculated, each guinea-pig receiving four drops subdurally from a fine needle.

1. Virus 1 c.c.+serum $\frac{1}{4}$ c.c.+salt sol. $\frac{3}{4}$ c.c.

Pig No. 1 well; November 30, discharged.

Pig No. 2, first symptoms of rabies November 22; dead November 23.

2. Virus 1 c.c.+serum $\frac{1}{2}$ c.c.+salt sol. $\frac{1}{2}$ c.c.

Pig No. 1 well; November 30, discharged.

Pig No. 2 well; November 30, discharged.

3. Virus 1 c.c.+serum 1 c.c.

Pig No. 1 well; November 30, discharged.

Pig No. 2 well; November 30, discharged.

4. Virus 1 c.c.+salt solution 1 c.c. (control).

Pig No. 1 died of rabies November 20.

Pig No. 2 died of rabies November 21.

From this test it is evident that this serum in the proportion of 1 to 4 of virus is hardly sufficient to kill all of the organisms, one of the pigs inoculated with this mixture dying of rabies with a prolonged incubation and the other escaping. That the killing effect in such a serum is due to specific properties in it, caused by the treatment, has been proved by numerous experiments with the sera of normal dogs, horses and rabbits, all of which sera are entirely without effect.

It has been difficult to prove that this serum obeyed the same laws, in its action as bactericidal sera, owing to the fact that the living animal must be used for its test. The serum inactivated by heat may be reactivated by the blood of the animal used in the test. This question will be studied in the future by applying the method of Bordet-Gengou.

4. The question as to whether the increase in immune body caused by the Pasteur treatment was confined to the blood serum alone or whether the central nervous system was involved gave rise to the following experiment:

A rabbit was treated at intervals from January 2 to April 15; on April 24 it was bled to death. A thick emulsion of the brain and cord was made and after standing, the supernatant fluid was pipetted off to be tested. The same was done with the brain and cord of a normal rabbit. The virus used was a thin emulsion of fixed virus. The virus and brain emulsions of the normal and treated rabbits were then mixed and like proportions of the virus and serum of the treated rabbit were kept under the same conditions. Guinea pigs were then inoculated as follows:

1. Brain emulsion (normal rabbit) 1 c.c.+fresh rabbit serum 1 c.c.+virus 1 c.c.

Pig No. 1 died April 30.

Pig No. 2 died May 1.

2. Brain emulsion (immune rabbit) 1 c.c.+fresh rabbit serum 1 c.c.+virus 1 c.c.

Pig No. 1 died April 30.

Pig No. 2 died May 1.

3. Serum of immune rabbit 1 c.c.+fresh salt solution 1 c.c.+virus 1 c.c.

Pig No. 1 discharged, well, May 16.

Pig No. 2 discharged, well, May 16.

4. Salt solution 2 c.c.+virus 1 c.c.

Pig No. 1 died April 30.

This experiment appears to indicate that the effect of immunization is to increase the immune body in the blood to such an extent that de-

velopment of the rabic organs in the nervous system does not occur, and not that the nervous system itself takes any active part. The above experiment is, of course, merely a rough indication, as the brain emulsions used may not have contained all the properties of the brain cells.

Concerning the loss of strength of the serum on standing, we have made the following observations:

Loss of strength of serum due to standing. The following observations have some bearing on these questions:

To test the loss of strength of sera kept in the ice box two samples of sheep serum were used, one which had been kept 1 month and the other $3\frac{1}{2}$ months. Both samples had been strong shortly after being drawn, acting successfully in the living animal.

The first sample showed slight action in dose of one-half as much serum as virus and full killing power in equal amounts.

The second sample showed no killing effect in dose of $\frac{1}{2}$ as much serum as virus and only feeble action when the two were used in equal amounts. The first sample was tested in animals and found to have little effect in prolonging incubation.

It would seem, then, that the serum should be freshly drawn, if possible, and probably not kept longer than two weeks. Further, that a number of animals should be constantly under treatment, so that while some are being inoculated others would be ready for bleeding. Further work must determine how a stronger serum is to be obtained from sheep or horses.

Action of the Serum in the Living Animal.

It was found that the injection of immune serum into rabbits gave disappointing results, the treated animals sometimes dying earlier than the controls. This was thought to be due to the fact that the serum was somewhat poisonous for the rabbits through a possible neurolytic action. The fact that rabbit's brain and cord tissue was injected into the animal furnishing the serum would account for this. Guinea pigs were therefore used. A further point noted with regard to guinea pigs was that they appeared to be poisoned by repeated doses of dog serum; the second injection frequently causing death, whereas the first was apparently without effect. This was thought to be due to a haemolytic

action of the dog's serum on the guinea pig's red cells. An example of this is as follows:

Two large healthy guinea pigs were inoculated as follows:

Pig No. 1—May 5 received $1\frac{3}{4}$ c.c. of normal dog serum subcutaneously; May 8 received 2 c.c. of normal dog serum; May 11 pig is sick; May 15 pig died.

Pig No. 2—May 5 received $1\frac{3}{4}$ c.c. of normal rabbit serum.

May 8th received 2 c.c. of normal rabbit serum.

April 1st pig was alive and well and discharged.

Another test—May 19th, large, healthy pig received $1\frac{1}{2}$ c.c. of normal dog serum.

May 21st, appeared normal and was given 1 c.c. of same serum.

May 23d, pig died.

The best combination was found to be guinea pigs inoculated with the serum of sheep or horses.

A further point to be noted is the necessity of employing a large number of animals in each experiment and the necessity of many experiments before any conclusion can be drawn. This follows from the well-known facts concerning the marked irregularity in incubation of rabies in guinea pigs when they are infected peripherally, as by inoculation into the leg. A number of animals inoculated in the same manner and with the same dose will come down with the disease at times varying a week or more and some may even fail to be infected at all. This is probably due to a variety of causes, e. g., the peculiar manner in which the infection travels, the probably protozoan nature of the organism, individual susceptibility of the animals, etc. It is, therefore, necessary in estimating the effect of an immune serum in lengthening the incubation in rabies, to employ several animals and take an average period of incubation in the treated animals and in the controls.

The following are some of the tests, made with immune serum in animals: March 30th, 15 pigs were inoculated in the region of the sciatic nerve with 1 c.c. of brain emulsion of street virus; 5 pigs were kept as controls, 5 were to be treated every day with 1 c.c. of a mixture of immune dog serum (1 month old) and fresh rabbit serum

(complement); and in a third set of 5 pigs, each was to receive a single injection of 2 c.c. of a mixture of dog and rabbit serum one hour after infection by the virus.

The average duration of incubation in the controls was $17 \frac{2}{5}$ days.

In the series receiving the repeated small doses of the serum, all became sick on April 4th after the second dose, and all but one died on that or the following day from the poisonous effects of the serum. This one survived and finally succumbed to rabies, May 7th, after an incubation of thirty-four days.

In the series receiving the single large dose of serum one was alive and well on May 19th and discharged on that date. One died accidentally, not of rabies. The other three died of rabies with an average incubation of 21 days.

Another test:

12 pigs were inoculated June 1st with 1 c.c. of street virus in the sciatic nerve.

6 pigs were kept as controls. These were given, two hours later, 2 c.c. of immune sheep serum. The remaining three were given on each of the three following days $\frac{1}{2}$ c.c. of the serum and on the fourth day 1 c.c.

The average incubation of the controls was $17 \frac{3}{5}$ days.

Of those receiving the single large dose of serum all contracted rabies with an average incubation of 29 days.

Of the three receiving repeated small doses, one died accidentally, not of rabies. One died of rabies with an incubation of 18 days and one was discharged alive and well six months later.

The attempt was next made to combine the immune serum with the Pasteur treatment to produce its full effect in immunization in man.

Thirty-six pigs were used, each inoculated in the leg with 1 c.c. of street rabies on August 4th. The animals were divided into six groups of six to a group.

In the first group a single large dose of serum (2 c.c.) was given $1\frac{1}{2}$ hours after the virus.

In the second the same dose of serum at the same interval and in addition the pigs received a short course of Pasteur treatment (10 days).

In the third group a smaller dose of serum (1 c.c.) was given late (24 hours after infection), and in addition the same Pasteur treatment.

In the fourth group a single dose of non-immune serum 1½ hours after infection.

In the fifth group, no treatment. Controls.

In the sixth group the Pasteur treatment alone.

On September 1st, 28 days later—

Of the 1st group 75% were alive.

Of the 2d group 66% were alive.

Of the 3d group 50% were alive.

Of the 4th group 50% were alive.

Of the 5th group 50% were alive.

Of the 6th group 25% were alive.

From this series it appeared (1) that the serum prolonged the incubation, (2) that the Pasteur treatment as given, acted as a poison and shortened the incubation, and that where the two were combined the good effect of the serum tended to counteract to some extent the bad effects of the Pasteur treatment.

It being evident that the Pasteur treatment could not be given in the ordinary way to guinea pigs, a mixture of the serum and fixed virus was used as suggested by A. Marie. Not being familiar at that time, with the details of the method, it was not given in strict accordance with Marie's directions.

It is evident that in the experiments above cited, the clinical conditions occurring in face bites in man are not correctly represented. In the latter case the short incubation is not necessarily due to a very large number of rabic organisms introduced into the body, but rather to the fact that they are introduced near the brain. In order to obtain a short incubation (15-20 days) in the guinea pig by inoculation into the leg with a brain emulsion virus, it is necessary as a rule to use a large amount of the virus representing an immense number of organisms. The great majority of these are soon taken up into the circulation and have little or no part in producing infection, those that remain behind and travel up the nerve track accomplishing this. The immune serum would nevertheless be partially used up in attacking these organisms in the circulation, and it would seem that it would be largely wasted in so

doing. If it were possible to use a small number of organisms which would nevertheless produce an infection with a short incubation when inoculated peripherally, the conditions which we have to treat in man would be more nearly represented.

From work which we have done with the salivary glands of rabid dogs it has seemed to us that while the saliva varied considerably in virulence, yet when a virus which was strong was obtained from the glands, it was apt to infect animals more readily than the brain emulsion when given peripherally. At any rate it is very probable that it contains fewer organisms than the brain emulsion, as the latter is the medium in which the organism grows. It is a fact that we have often given as much as a c.c. of a thick brain emulsion in the leg of a guinea pig, and seen the disease produced very slowly or even not at all. On the other hand, we have produced the disease with a very short incubation with $\frac{1}{2}$ c.c. (or even less) of either a salt solution extract or a glycerin extract of the salivary glands of the same dogs.

In the following experiment, carrying out the above ideas, we used as a virus $\frac{1}{2}$ c.c. of a salt solution extract of the salivary glands of a dog with street rabies, and treated one-half the pigs with a mixture of immune serum and fixed virus vaccine as follows:

November 27th six pigs were inoculated in the region of the sciatic with $\frac{1}{2}$ c.c. of gland virus. Three were kept as controls, and each of the other three were given subcutaneously half an hour after infection 2 c.c. of the following mixture:

Immune horse serum (heated to 60 deg. for $\frac{1}{2}$ hr.), 8 c.c.

Fresh horse serum, 4 c.c.

Fixed virus brain emulsion, 4 c.c.

Again on December 4th each of these pigs received 4 c.c. of the following mixture:

Immune horse serum (heated to 60 deg.), 8 c.c.

Fresh horse serum, 4 c.c.

All the pigs died of rabies, the average incubation of the controls being 10 $\frac{2}{3}$ days, and of the treated pigs being 29 $\frac{2}{3}$ days.

By using sheep instead of guinea pigs and combining the serum and virus in a manner recommended by Marie, Remlinger has been

able to protect his treated animals completely against an intravirulent infection of rabies when the latter was given 3 days before the injection of serum virus mixture.

Another experiment was undertaken to compare the amount of immune body in the blood of two sheep at the end of seventeen days after the beginning of treatment, the one sheep having been treated by the ordinary Pasteur method, the other by injections of mixtures of virus and serum. A preliminary test of the serum of the sheep made before the beginning of treatment showed both to be entirely inactive in the proportions used in the subsequent tests made at the end of treatment.

Treatment begun December 14th.

One sheep (No. 1) received the ordinary Pasteur treatment for 17 days.

The other (No. 2) received December 14—Immune horse serum, 8 c.c. (4½ weeks old); fresh horse serum, 4 c.c.; fixed virus brain emulsion, 4 c.c. N. B.—This mixture was neutral.

December 19—Immune horse serum, 12 c.c.; fresh horse serum, 5 c.c.; fixed virus brain emulsion, 9 c.c.;

December 26—Immune horse serum, 15 c.c.; fresh horse serum, 8 c.c.; fixed virus brain emulsion, 15 c.c.

On January 1st both sheep were bled and the serum tested as follows:

Sheep No. 1 (Pasteur treatment):

1. Virus, ½ c.c.+serum, 2 c.c.

Pig No. 1 died 1/7 a. m.

Pig No. 2 died 1/7 a. m.

2. Virus ½ c.c.+serum 1 c.c.+salt solution 1 c.c.

Pig No. 1 died 1/6.

Pig No. 2 died 1/6.

3. Virus ½ c.c.+serum ½ c.c.+salt solution 1½ c.c.

Pig No. 1 died 1/7 a. m.

Pig No. 2 died 1/6.

Sheep No. 2 (serum virus mixture):

1. Virus ½ c.c.+serum 2 c.c.

Pig No. 1 died 1/8 a. m.

Pig No. 2 died 1/7 a. m.

2. Virus $\frac{1}{2}$ c.c.+serum 1 c.c.+salt solution 1 c.c.
 Pig No. 1 died 1/7 p. m.
 Pig No. 2 died 1/8 a. m.
3. Virus $\frac{1}{2}$ c.c.+serum $\frac{1}{2}$ c.c.+salt solution $1\frac{1}{2}$ c.c.
 Pig No. 1 died 1/7 a. m.
 Pig No. 2 died 1/7 a. m.

From this experiment, we see that by neither method of treatment is immune body produced to any extent in so short a time as 17 days, but that what slight difference there is, is in favor of the serum virus mixtures.

Conclusions.

From the above experiments one may conclude:

1. That it is possible to produce a strong immune serum against rabic virus, in rabbits, sheep, dogs and horses.
2. That for the production of such a serum a long course of treatment is necessary.
3. That this serum when fresh prolongs the incubation of rabies when injected into test animals a short time after infection.

Further work must be done to determine the method of combining the serum and virus to produce the best results. It is expected that in the near future we shall fully test Marie's method of preventive inoculation in dogs. We have at present three sheep under treatment for the production of serum and are prepared to employ this in combination with the Pasteur treatment in such cases as warrant its use.

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THE AGGLUTINATION TEST AS APPLIED TO THE DIAGNOSIS OF GLANDERS.

(Preliminary Summary.)

By K. R. Collins, M. D., Bacteriologist.

The readiness with which the agglutination test can be carried out when compared to the mallein test for glanders in horses has made it the subject of much investigation by those interested in comparative medicine.

Pokchichevski, in 1902, states that normal horse blood will agglutinate the *B. mallei* in dilutions of 1:300, while the sera of horses infected with glanders will agglutinate it in dilutions of 1:500 and above.

Afanassjeff shows practically the same results. Schütz and Meissner from the Pathological Institute, Berlin, 1905, undertook an extensive series of observations upon the subject.

A number of normal horses were given experimental glanders and in from six to twelve days the agglutinating index of the blood serum of these horses had increased from 1:300 to 1:2000 and 8000. Subsequent autopsy of these horses showed the typical lesions of glanders in the lungs and other organs.

In the course of their work 2,209 horses were tested. These consisted of horses that were normal, those ill of other diseases than glanders, and those having glanders. The three accompanying tables give the average results of their findings:

TABLE I.

Free from Glanders.						Glanders.					
1-300	400	500	600	800	1000	500	600	800	1,000	1,500	2,000
145	36	17	15	6	2	2	4	6	11	6	5
221 (25 killed).						34					

255 horses.

TABLE II.

Free from Glanders.						Glanders.					
1-300	400	500	600	800	1,000	500	600	800	1,000	1,500	2,000
5	4	2	1	4	1	..	2	2	4	9	2
17						19					

36 horses.

TABLE III.

Disease.	100	200	300	400	500	600	800	1,000
Drüse (glands or strangles)	1	3	1
Catarrh of superior maxillary sinuses.....	..	1	1	1	1	2
Lymphangitis.....	..	1	2	1	1
Various skin diseases.....	..	1	..	1	1	..
Lymphosarcom.....	..	1	1	1	1	..
Pleurisy	1	2	1
Foci in the lungs	2	4	4

Schnürer and Bonome verified these results. Hutyra, of Budapest, in experimental glanders in horses found that the agglutination index raised from 1:100 to 300 before injection to 1:1000 to 1:2000 several days after inoculation.

Moore, Taylor and Giltner found the index of normal horses tested to range from 1:100 to 1:500; while in twelve horses with diseases other than glanders, the index did not exceed 1:500, and in thirty-seven horses suspected of having glanders, all cases reported as positive by the veterinarians in charge, agglutinated in dilutions of 1:600 and above.

For the past several months the work carried on in this laboratory has been for the purpose of ascertaining to what extent this test can be relied upon in the diagnosis of glanders.

Since August 14, 1905, the sera from four hundred and fifty-seven horses have been tested. In many instances a series of observations have been made from time to time upon the same horse. We are indebted to Dr. H. D. Gill, Veterinarian for the State Department of Health, and to Dr. A. D. Silkman, Veterinarian for the Health Department of New York City, for the collection of sera, clinical observations and autopsies. Dr. R. H. Kingston, Veterinarian of New York City, has also aided us in supplying cases other than glanders that have been of interest and value in the work.

In making the test for agglutination a culture of *B. mallei* has been used which was obtained from Dr. Moore, of Cornell University. Other cultures obtained from different sources (two from human cases) have been tested from time to time, but the one culture, as stated, has been relied upon throughout as a standard culture.

Schütz and Meissner state that in order to obtain a culture whose agglutinability does not vary the organism must be passed through an animal every two weeks. This assertion is in direct contradiction to our experience with other organisms, it being a well-known fact that recently isolated dysentery and typhoid bacilli are poor agglutinators, but after long growth upon artificial media may have their agglutinability increased. The possibility of this being due to the contact of the organisms with the serum elements of the body (which, as has been shown, may contain some normal agglutinins) is indicated by the experience of Marshall and Knox and Park and Collins. These authors demonstrate that dysentery bacilli and typhoid bacilli grown for some time in the presence of a specific serum, may lose their agglutinability, and that this loss can be recovered after a long period by the withdrawal of the serum from the media. Bearing in mind, however, that this might not be true for the *B. mallei*, the original culture has been controlled from time to time by one recently passed through the guinea pig; thus far no difference has been noticeable excepting that which may be attributable to other conditions.

Hanging drops have been chiefly relied upon, these being frequently controlled by the tube method. The *B. mallei* forms in 24 hours a light growth which gives about the right number of bacilli for testing with the hanging drop, but not sufficient to be recognized macroscopically; hence a much heavier growth (about 3 days) is requisite for the latter method. As old cultures are liable to lose their agglutinability to some extent, the younger cultures are preferable and more reliable, therefore if the tube method is used, an emulsion of the bacilli grown on agar is best. One advantage of the hanging drop over the tube method is that contaminations and anomalous conditions that have sometimes been met with are quickly detected. Federowsky has also found the microscopical method to be the more delicate and reliable.

The hanging drop controlled by the tube with a young culture shows no practical difference examined microscopically. The hanging drop where the young culture is used and the tube where the older culture is used will show a difference due both to the diminished agglutinability of the old culture, and to the greater number of organisms in

the latter. Where the old culture is used the index will therefore be lowered.

The twenty-four hours' broth culture is heated to 60° C. for one minute, this proving sufficient to check the growth of the bacteria and yet not affecting their agglutinability.

Clumping does not occur as quickly as with some other organisms such as the typhoid and dysentery bacilli, but, like the pneumococcus, requires from twelve to eighteen hours for completion.

In October we tested the blood of thirteen horses from a stable where a case of glanders had developed a few weeks previously. Mallein was then given to eight of them. Nine days later two of the number were tested again for the agglutination reaction and with mallein. Four days later blood tests were again made for the three.

The following table gives the result of these tests:

TABLE IV.

	October 30.		November 8.		November 12.	
	Agglutination.	Mallein Reaction.	Agglutination.	Mallein Reaction.	Agglutination.	Mallein Reaction.
1.....	5,000	Neg.				
2.....	2,000	Neg.				
3.....	200					
4.....	2,000	Neg.				
5.....	1,000	Good.	10,000	Positive.	20,000	*
6.....	5,000					
†7.....	200					
8.....	2,000	Partial.				
9.....	3,000	Partial.				
10.....	5,000	Partial.				
11.....	1,000					
12.....	2,000	Good.	10,000	Good.	10,000	*
13.....	2,000					

* These were destroyed because of the glanders infection.

† Died of pneumonia.

Unfortunately autopsies could not be obtained in these cases on account of lack of facilities, and we have not been able to make further observations upon this stable.

Bonome and others claim that the agglutination reaction increases after the administration of mallein. This, however, has not been my experience, and the above table shows in two horses a decrease after mallein, while in the two horses that died the increase in index may well be attributed to the progress of the disease, as the repeated mallein reaction follow the same curve.

The blood of thirty horses from a stable where glanders had existed was tested and mallein was given in six cases one month later. The results were as follows:

Three horses agglutinated at 1:5000, one of the three tested a month later with mallein gave no reaction, one died of glanders. Two agglutinated at 1:2000 and were negative to mallein. Two agglutinated at 1:1000 and one tested with mallein gave a negative reaction. Four agglutinated at 1:500. One that agglutinated at 1:200 gave a slight reaction to mallein a month later. One agglutinating at 1:200 was negative to mallein, the remainder agglutinated at 1:200 and below and were not tested with mallein.

TABLE V.

The sera from fifty horses taken from a stable where glanders had occurred, agglutinated as follows:

9	agglutinated at 1:500	3	tested with mallein,	2	reacted.
9	" " 1:1000	6	" " "	3	"
7	" " 1:2000	4	" " "	4	"
25	" below 1:500	2	" " "	2	negative.

In sera from thirty-two horses from another stable where glanders had occurred were tested the same as above.

4	agglutinated at 1:500	2	reacted to mallein.
12	" between 1:1000 and 1:2000	6	" " "
7	" " 1:5000 and 1:10000	3	" " "
9	" below 1:500	0	" " "

Three normal mules tested gave the following reactions:

1. Reacted at 1:1000.
2. Reacted at 1:1000.
3. Reacted at 1:1000.

One mule condemned for glanders on physical signs gave a reaction in 1:20000. The normal agglutinins present in the blood of the mule may perhaps show a higher average than in horses.

A series of tests made upon four horses show the variation in the agglutination reaction that may sometimes occur. A regular increase is observed in three while the third shows an irregular rise and fall. This condition occurs constantly in experimental animals immunized with various organisms.

No. 1. *Glanders*—Agglutinated at 1:500, mallein positive; later agglutinated at 1:2000, mallein positive; later agglutinated at 1:10000, mallein positive.

No. 2. *Glanders*—Agglutinated at 1:5000, mallein positive; later agglutinated at 1:10000, mallein positive.

No. 3. *Suspicious*—Agglutinated at 1:2000, mallein good; five weeks later agglutinated at 1:5000, mallein good; ten days later agglutinated at 1:2000, mallein good; two days later agglutinated at 1:5000, mallein good.

No. 4. *Suspicious*—Agglutinated at 1:20000, mallein good; five weeks later agglutinated at 1:5000, mallein good; five weeks later agglutinated at 1:5000, mallein good.

From a stable of horses that were supposed to be normal the sera from seven agglutinated below 1:500; three agglutinated at 1:500; four agglutinated at 1:1000 and above.

One horse with an index below 1:500 and one with an index above 1:500 were tested with mallein and both gave a negative reaction. This stable continues under observation.

One horse from this stable reacted at 1:200; four months later a test was made and the index was 1:5000. The horse at this time had two ulcers on the legs. An organism recovered from the pus morphologically resembled glanders and was quite virulent for guinea pigs

but failed to develop agglutinins for glanders in the animal or to give any of the other reactions peculiar to the *B. mallei*.

Schütz and Meissner found that the blood of one horse with lymphangitis out of four such horses tested, agglutinated at 1:1000, and two at 18:00; they state that this is the only condition not glanders that will give such a high reaction.

Nine horses with lymphangitis were tested by us and mallein given to four.

Two agglutinated at 1:2000; mallein not given.

One agglutinated at 1:1000; mallein not given.

One agglutinated at 1:500; mallein not given.

One agglutinated at 1:100; mallein not given.

One agglutinated at 1:5000; mallein negative.

One agglutinated at 1:1000; mallein negative.

Two agglutinated at 1:2000; mallein positive.

The question that might arise here is whether or not there was a latent glanders in those cases where the reaction is high. More extensive research is necessary before any conclusion can be reached, but the fact that two horses, apparently healthy, reacted to mallein is suggestive. The mallein was not given until the horses had fully recovered from the attack of lymphangitis.

In one stable of thirteen horses the sera were tested for the agglutination reaction; mallein was given and then the horses were killed and autopsied. In all but one instance the results of the agglutination reactions were confirmed by the mallein test and autopsy.

TABLE VII.

The following list of horses was tested for the agglutination and finally killed because of glanders:

Condition Reported.	Agglutination.	Mallein.	Disposition.
1. Enlarged submaxillary	5,000	Destroyed.
2. Eruption	500	"
3. Septic condition	1,000	"
Sixteen days later.....	5,000	Positive	"
4. Farey	2,000	Positive	"
5. Suspicious	1,000	"
6. Continued high temperature.....	1,000	"
7. Enlarged submaxillary.....	5,000	"
8. Suspicious	2,000	"
9. "	10,000	Positive	"
10. Glanders	20,000	Positive	"
11. Ulcer of septum.....	5,000	Negative	"
12. Mule glanders.....	20,000	"
13. Nasal discharge.....	1,000	"
14. Glanders	2,000	"
15. Suspicious	2,000	Good	"
16. "	2,000	Positive	"
17. "	2,000	Good	"
18. "	500	"
19. "	2,000	"
20. Continued high temperature for weeks	20,000	but no report made.	
21. Suspicious	500	Good	Destroyed.
22. Enlarged submaxillary.....	1,000	"
23. Suspicious	5,000	"
24. Glanders	2,000	"

The following list is comprised of horses that were tested with mallein and for the agglutination reaction. The horses are still working:

TABLE VIII.

Condition.	Agglutination.	Mallein.
1. Glanders	5,000	Positive
2. Enlarged submaxillary.....	5,000	Good
3. Suspicious	2,000	Good
4. Enlarged submaxillary.....	10,000	Good
5. Eruption	2,000	Negative
6. Suspicious	5,000	Negative
7. Exposed to glanders.....	500	Negative
8. Suspicious	10,000	Good
9. "	2,000	Good
10. "	5,000	Positive
11. "	5,000	Good
12. "	5,000	Positive
13. "	5,000	Positive
14. "	2,000	Good
15. "	2,000	Good
16. "	500	Negative
17. "	1,000	Positive
18. "	2,000	Positive
19. "	2,000	Negative
20. "	2,000	Questionable
21. "	2,000	Good
22. "	10,000	Good
23. "	1,000	Good
24. "	200	Negative
25. "	Negative	Negative

The following list consists of horses tested for the agglutination reaction only :

TABLE IX.

Condition.	Agglutination.
1. Swollen leg	1,000
2. Suspicious	500
3. Absces on hip.....	1,000
4. Suspicious	10,000
5. "	2,000
6. "	500
7. "	1,000
8. Abscess over eye.....	5,000
9. Suspicious	500
10. "	1,000
11. "	500
12. "	2,000
13. "	5,000
14. "	10,000
15. "	500
16. "	1,000
17. "	2,000
18. "	200
19. "	negative
20. "	5,000
21. "	2,000
22. "	5,000
23. Enlarged submaxillary	5,000
24. Suspicious	5,000
25. "	1,000
28. "	2,000
29. "	5,000
30. "	10,000
31. "	1,000
32. "	negative
33. "	1,000
34. "	2,000
35. Pyaemia	200(died)
36. Chronic catarrh	200
37. Blood disorder	100

TABLE IX (Concluded)

Condition.	Agglutination.
38. Enlarged submaxillary	100
39. Azoturia	2,000
40. Infected muscle	negative
41. Maxillary abscess	negative
42. Nasal discharge	500
43. Normal	100
44. "	100
45. "	200
46. "	200
47. "	negative
48. "	200

Summary.

It is not possible to draw definite conclusions as to the specificity of the agglutination reaction in glanders until the findings in questionable cases have been confirmed by careful autopsies.

The facts are suggestive, however, and tend to substantiate the views of all who have investigated the subject that high reactions indicate the presence of glanders, and reactions even as low as 1:500 must be looked upon with suspicion. Further research will help to establish the reliability of the reaction, careful autopsies and systematic observation of the same horses over an extended period of time being the requirements best calculated to furnish definite and satisfactory provings.

At present the value of the test lies in its use as an indicator for the mallein test. In our hands, since the technique has become uniform, only one horse, as far as tested, has failed to give an agglutination reaction where it has responded to the mallein test; as a second testing was not practical at the time, this one negative case with so many positive cases becomes a negligible quantity.

The presence of the agglutination reaction before the mallein reaction is accounted for by the fact that agglutinins are, as a rule, very quickly raised in the animal body, other antibodies oftentimes not appearing until late. A slight infection would then be sufficient to in-

duce this reaction. This has been shown with some horses, which gave only an agglutination reaction at first. Several weeks later they showed an increase in the agglutinating index and reacted to mallein and then either exhibited the physical signs of glanders before death or the characteristic lesions on autopsy.

A few human cases of glanders have been studied. The bloods of these patients have agglutinated in dilutions of 1:1000 and 1:2000. In two cases the *B. mallei* has been recovered, from the pus of an abscess in one case, and from the blood in a second case.

The sera from patients having diphtheria, scarlet fever and tuberculosis have been tested with the *B. mallei*. In some instances they have agglutinated the bacillus in dilutions of 1:50, and once as high as 1:100. In cases of glanders, as far as tested, the reactions occur in much higher dilutions so that the chances for confusion of any of these conditions with glanders, at least as far as the agglutination reaction is concerned, is very slight. In all cases of apparently irregular typhoid the blood should be tested with the *B. mallei*.

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[The completed study will appear in the 1907 volume of the Studies from the Research Laboratory.—Editor.]

AGGLUTININS AS RECEPTORS OF THE THIRD ORDER.

KATHERINE R. COLLINS, M. D., Bacteriologist.

The following limited number of experiments were undertaken for the purpose of determining to what extent the results of the work on agglutinins being carried out at the time, might be affected by the more recent views, advanced by some observers, that agglutinins may sometimes be receptors of the third order and require the addition of complement or a corresponding substance to bring about a reaction.

Joos believes that the agglutination reaction resembles the formation of a double salt, thus requiring the presence of a third substance. Bail places the agglutinins in the third order, while Eisenberg and Volk are uncertain whether they are in the second or third. Formerly Ehrlich believed agglutinins to be of the second order but has in a recent general review on the work on immunity suggested the possibility of the precipitins and agglutinins behaving at times in a similar manner to the immune bodies.

Muir and Browning in the course of some haemolytic experiments found that agglutination of the stromata occurred upon the addition of a complemental serum. This agglutination passed off after several hours, but partially returned upon further addition to the fresh serum. This led the authors to the view that agglutinins might sometimes be receptors of the third order; they state, however, that they made only one observation.

The foregoing opinions have been based chiefly upon results obtained from red blood cells. Ohwada, however, found that an old typhoid serum which had lost its agglutinating power was reactivated by the addition of fresh normal rabbit serum, but not by fresh horse, dog, guinea pig, cat, or chicken serum. He names the substance in the fresh serum "add-agglutinins."

The following experiments have given negative results in every combination except where the amount of complemental serum added would alone agglutinate the organism used:

Serum of a Horse Immune to B. Typhi to which Fresh Normal Horse Serum Has Been Added and Tested with B. Typhi.

Index of Immune Serum before Treatment.	Index of Fresh Normal Horse Serum.			Index of Immune Serum + B. typhi; Normal		Serum + Fresh B. typhi & Once.
	1:10	1:20	1:50			
1:1000				1:1000	1:1000	1:1000
++	—	—	—	++	++	++

Same Index as Above.	Index of Fresh Normal Horse Serum.			10.		Immune Serum + Fresh Normal Horse Serum + B. typhi Added at Once.		
	1:50					1:1000	1:2000	1:5000
1:1000								
++	++	++	++	++	++	++	++	++

The same immune serum as above, heated to 56 degrees for 30 minutes, shows a diminution in the amount of agglutinins which the addition of fresh normal horse serum does not restore.

The serum of a second horse immune to B. Typhi was tested in the same manner with fresh normal rabbit serum added as complement; the results were the same as above.

A rabbit serum immune to the B. Dysentery (Shiga Type), heated to 56 degrees for 30 minutes, and fresh normal rabbit serum used as complement, also failed to show increase or restoration of agglutinins.

Finally a rabbit serum immune to B. Dysentery, two years old and showing partial loss of agglutinins, was tested, fresh normal rabbit serum being used as a complement. Here again the results were negative.

Since this paper was written Shibayama has made a series of tests with immune horse serum heated to varying degrees for different periods of time and to which he added fresh normal rabbit serum in amounts above the normal index of the serum. In this way he restored to the immune serum the agglutinins that had been destroyed by heat. These results I have failed in repeated instances to bring about, and where changes do occur they would seem to me to be purely of a physical nature. I cannot share the rather more prevalent view, that the agglutinin in such instances acts as a receptor of the third order, according to Ehrich.

Conclusions.

The above experiments are not sufficient in number or latitude to refute, in the case of bacterial agglutinins, the findings of the several observers, that agglutinins may act like receptors of the third order. They demonstrate, however, that the phenomenon is not frequent and they suggest several possible sources of error that might readily lead to erroneous conclusions.

1. The technique of the agglutination reaction, as it stands at present, is of necessity variable within narrow limits, but uniform in the wider readings; hence, if the yearlings are taken within too narrow limits the results may be misleading.

2. The possibility of the normal serum containing agglutinins for the organism tested must be kept very definitely in view.

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SOME STATISTICS ON PNEUMONIA.

By Dr. CHARLES BOLDUAN, Bacteriologist.

The following data are compiled from the history cards filled out by the medical inspectors, for cases of pneumonia reported from the hospitals in New York City (Boroughs of Manhattan and Bronx) during the winter of 1904-5. There were 1,384 such cases, but on about 500 cards there were practically no data whatever, leaving about 900 cards as a basis of these observations.

Type of Pneumonia.

There were 776 cases of frank lobar pneumonia, with 206 deaths— $26\frac{1}{2}$ per cent. mortality. There were 119 cases of broncho-pneumonia, with 41 deaths— $34\frac{4}{10}$ per cent. mortality.

Age Incidence.

In 876 cases of pneumonia there were sufficient data to learn something of the age incidence of the disease, especially in a comparison of the lobar type with the broncho type. Of these 876 cases, 760 were lobar pneumonia, and 116 were broncho pneumonia, a proportion of about $6\frac{1}{2}$ to 1.

The type of the disease at different ages is well shown by the following tables:

TABLE 1.

This gives the actual number of cases reported for the age period stated.

Lobar Pneumonia.

Under 1 Year.	1 Year and Over, but Under 5.	5 and Under 10.	Total Under 10.	10 and Under 15.	15 and Under 20.	Total 10-20.	20-30.	30-40.	40-50.	50-60.	60-70.	70-80.	Over 80.	Totals.	Propor- tion.
13	43	28	84	20	52	72	178	177	131	75	32	9	2	760	84.5%

Broncho Pneumonia.

23	47	14	84	2	3	5	7	5	5	1	3	5	1	116	15.5%
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TABLE 2.

This shows the percentage, of the cases at the age group, on the total number of cases of that type.

	Under 1 Year.	1 Year and Over, but Under 5.	5 and Under 10.	Total Under 10.	10 and Under 15.	15 and Under 20.	Total 10-20.	20-30.	30-40.	40-50.	50-60.	60-70.	70-80.	Over 80.	Totals.
Lobar ...	1.7	5.6	3.7	11.0	2.6	6.8	9.4	23.4	23.2	17.2	9.8	4.2	1.2	0.3	100%
Broncho.	20.0	40.0	12.0	72.0	2.0	2.5	4.5	6.0	4.3	4.3	0.9	2.5	4.3	0.9	100%

An examination of these tables brings out a number of interesting points. Thus, while the ratio of lobar pneumonia to broncho pneumonia generally, is about $6\frac{1}{2}$ to 1, the proportion at the different age groups presents certain marked variations. Thus under one year, there are almost twice as many broncho pneumonias as lobar. Between one and five years, the figures are about equal, and commencing with five years, the lobar pneumonias begin to outnumber the bronch pneumonias. In the decennial periods 20/30, 30/40 and 40/50, there are more than twenty times as many lobar pneumonias as broncho pneumonias. Finally, in the very old people, we see the relative proportion between the two types approach that of the early periods of life.

If we regard each form of pneumonia separately, as is done in Table 2, we see that lobar pneumonia, when it occurs, is most apt to attack individuals between 15 and 60, preferably between 20 and 40. Almost 50 per cent. of the cases of lobar pneumonia fell in the latter age group.

When broncho pneumonia, on the other hand, occurs, our table shows that over 70 per cent. of the cases are in children under ten years old.

Mortality.

The termination of the two varieties of pneumonia at the various age groups is well shown in the following table:

TABLE 3.

	Under 1 Year.	1 Year and Over, but Under 5.	5 and Under 10.	Total Under 10.	10 and Under 15.	15 and Under 20.	Total 10-20.	20-30.	30-40.	40-50.	50-60.	60-70.	70-80.	Over 80.
<i>Leber.</i>														
Recovered	9	35	28	72	17	44	61	136	137	79	36	13	3	1
Died.....	4	8	..	12	3	8	11	42	40	52	39	19	6	1
Per cent. mor- tality in each age group....	31%	18%	..	17%	15%	15%	15%	24%	23%	40%	52%	60%	66%	50%
<i>Bronche.</i>														
Recovered	11	29	13	53	2	3	5	7	5	3	1	..	1	..
Died.....	12	18	1	21	2	..	3	4	1
Per cent. mor- tality in each age group....	53%	38%	7%	28%	40%	..	100%	80%	100%

As was stated above, the general mortality was $26\frac{1}{2}$ per cent. in the lobar pneumonia, and $34\frac{4}{10}$ per cent. in the broncho pneumonia. The table just given, shows that in lobar pneumonia the mortality is lowest in the five to ten-year period, and that from that time on, it gradually rises with the age of the patient. The same increase is noted as the age of the patient *decreases* from this five-year period, so that in the first year of life the mortality is over 30 per cent. In broncho pneumonia conditions are similar, though the mortality in the young is almost double that of the lobar type of the disease.

Site of Infection.

The site of infection is shown on 895 cards, and was as follows:

Lobe Involved.	Per Cent. of Whole Number.	Total Number.	Died.	Recovered.	Mortality in Per Cent. for That Lobe.
Upper right.....	12.8	99	16	83	16.2
Upper left.....	6.4	49	12	37	24.5
Lower right.....	18.3	142	36	106	25.3
Lower left.....	23.3	181	42	139	23.2
Other sites (usually several } lobes affected)..... }	39.2	305	100	205	33.
	100.	776	206	570	26.5
Broncho pneumonia.....	119	41	78	34.4
	895	247	648

Of the total number of cards returned, 489 do not show the site of infection. About 13 per cent. of the cases reported were broncho pneumonia. Whether this really represents the true proportion in which the disease prevailed during the season 1904-5, is impossible at this time to say, as we do not know whether the hospital authorities understood that *all* cases of pneumonia were to be reported to the Health Department, and not merely cases of lobar pneumonia.

It will be noticed in the above table that infection of the right lobe is associated with a low mortality. This is probably due largely to the small size of this lobe, and the consequent smaller extent of the infection.

So far as the site of the infection is concerned, it may be of interest to compare the findings with those of other authors. The following figures were obtained by Juergensen in a study of over 6,000 cases of pneumonia.

Upper right.....	15.15%
Upper left.....	6.96%
Lower right.....	22.14%
Lower left.....	22.73%
Other sites.....	33.02%

It will be seen that the New York cases (1904-5) show slightly smaller frequency of the upper and lower right lobes, but on the whole the correspondence is rather striking.

Nativity.

The nativity of the Manhattan and Bronx cases is as follows:

United States, white.....	465 cases, or 53%
United States, colored.....	58 " " 6½%
Irish	136 " " 14%
German	64 " " 7%
Italian	52 " " 6%
Russian	32 " " 3½%
English	19 " " 2%
All others.....	72 " " 8%
	<hr/>
	898 100%

In order to compare these cases with each other, we must know the nativity distribution of the population of Manhattan and Bronx. According to the United States Census of 1900, this was then as follows:

Total population Manhattan and Bronx, 2,050,600, of which 850,000 were foreign born.

This leaves born in the United States.....		1,199,716, or	58.5%
The foreigners were: {	Germans	189,720	" 9.0%
	Irish	178,886	" 8.5%
	Italians	103,795	" 5.0%
	Russian	129,700	" 6.0%
	All others.....	248,783	" 13.5%

In the population, the Germans slightly outnumber the Irish, yet the incidence of the pneumonia cases shows twice as many Irish as Germans. What are we to conclude from these figures? Are the Irish more than twice as susceptible to pneumonia as the Germans? Or are the occupations such, that the Irish, as a rule, are more exposed to the weather than are the Germans? Or, since these are all hospital cases, do the Irish seek hospital treatment more readily than the Germans? Some light is shed on this problem by a study of the nativity incidence of all deaths in Manhattan and Bronx. This is shown in the following table for 1902:

United States.....	25,876, or 62.0%
German	3,838 " 9.2%
Irish	5,286 " 12.7%
Italian	1,507 " 3.6%
Russian	1,212 " 2.9%

The distribution of the population as given above was: United States, 58 per cent.; German, 9 per cent.; Irish, 8½ per cent.; Italian, 5 per cent.; Russian, 6 per cent.

From this it would appear that the Irish do not seek hospital treatment any oftener than the Germans, for we see that the percentage of deaths among the Irish is here also greater than their relative strength in the population. Another fact emerges from this table when taken in conjunction with that showing the nativity incidence of the pneumonia deaths. In the latter the deaths among the Italians is greater than their share in the population; in the table of the total deaths, however, the Italians have less than their quota as indicated by the population. In other words, these people have also suffered more from pneumonia than have the native-born Americans or the Germans. This strongly supports the view that the greater prevalence of this disease among the Irish and the Italians is due to the more usual occupation of these people. In New York, at the present time, the Irish are largely employed as truck, cab, or car drivers, motormen, bricklayers, etc., while the Italians are very largely laborers. All these occupations are attended with considerable exposure to inclement weather.

Complications.

The most frequent complications in this series of cases were pleurisy with effusion, and empyema. Of the former, the cards record twenty-four cases; of the latter twenty-three cases. It is impossible, however, from these figures to calculate even approximately, the frequency of these complications, as a large number of cards have the heading "complications" not filled out. Whether this means that there were none, or that they were merely not noted, cannot be determined.

The twenty-four cases of pleurisy with effusion were made up of fifteen males, and nine females. Their age distribution is shown in the following table:

Years: 16†-18-18†-20-20-22-23-26-28-29-30-32-34-35-36-38-38-40†-40-42-44-47-54.

Of these, the cases marked with a dagger, were fatal; that is, three out of twenty-four died.

The cases complicated by empyema were twenty-three, of which twenty were males and three females.

Their age distribution was as follows:

Years: ½-2†-4-5-8†-12-18-19-21-22-23-27-28-30†-34†-34-38-38-47-48†-48-52-59.

Of these the cases marked with a dagger, were fatal. That is, five out of twenty-three died.

So far as the sex incidence of these cases is concerned, it does not differ materially from that of the pneumonia cases in general, which was, out of 974 cases, males 675, and females 299.

Attention may perhaps be called to one point of interest in connection with these complications, and that is the frequency with which empyema occurs in children, while pleurisy with effusion seems to occur only rarely at that time. However, the figures are really too small to draw any general conclusions.

THE FRACTIONAL PRECIPITATION OF ANTITOXIC SERUM.

Drs. E. J. BANZHAF and R. B. GIBSON.

Comparatively little attention has been paid to the fractional precipitation of antitoxin. Brodie¹ in 1897 separated antitoxic horse serum into four fractions by the progressive addition of ammonium sulphate to half saturation; all four contained, however, relatively equal amounts of antitoxin. Atkinson,² in the Research Laboratory of the Department, saturated with sodium chloride a solution of the moist serum globulin precipitate obtained with magnesium sulphate, and by the employing heat, differentiated the globulin into several fractions containing antitoxin. The protective properties corresponded roughly to the quantities of serum globulin in the precipitates. In some unpublished experiments he found that alterations of the amounts of coagulated proteid in the several fractions resulted if more magnesium sulphate was added before heating; there were proportionate changes in the distribution of the antitoxin. Owing to the destruction of a portion of the antitoxin at the higher temperature and possible injury by exposing it to heat of less degree, this fractionation must be considered as incomplete and does not exclude a purification of the antitoxin by salt fractionation. The work of E. P. Pick on the ammonium sulphate fractioning of the antibodies has been referred to in a preceding communication. Our own experiments have resulted somewhat differently from either those reported by Atkinson or by Pick, and have developed some important new and suggestive facts.*

On the basis of the solubility of the antitoxin proteids in saturated NaCl solution, one of us (Gibson) recently devised a method for the partial purification and concentration of antitoxin. This consisted in

¹Brodie: *Journ. of Path. and Bact.*, iv, p. 460, 1897.

²Atkinson: *Journ. of Exper. Med.*, v, p. 67, 1901.

*The literature on the purification and chemical characters of antibodies has been briefly reviewed in a paper on the "Practical Concentration of Diphtheria Antitoxin for Therapeutic Use," in the Report of the Department for 1905.

precipitating the diluted plasma with an equal volume of saturated ammonium sulphate and separating the antitoxin proteids by extracting the precipitate with saturated sodium chloride solution. We now have employed the method of salt fractionation to study further the concentration of antitoxin.

Twenty liters of plasma (475 units per c.c.) were diluted with 20 liters of water; by fractioning with ammonium sulphate the three proteid precipitates were obtained which separated at concentrations corresponding to 3.3 c.c., 3.3-3.8 c.c. and 38-50 c.c. of the saturated salt solution in 10 c.c. The NaCl soluble (antitoxic) globulins of these fractions and of the 5.0 saturation precipitate of a second 20 liters of the plasma were prepared as usual.

Prep. 77.

Fractions.	A. 0.0-5.0	B. 0.0-3.3	C. 3.3-3.8	D. 3.8-5.0
Volume, c.c.	5,200	1,440	1,400	2,050
Units per c.c.....	1,450	1,150	1,350	1,750
Times concentrated	3.05	2.42	2.84	3.68
Per cent. recovered.....	79.3	17.4	19.9	37.8
Proteid, gms. per 100 c.c.....	11.66	11.51	9.87	9.70
Units, per gm. proteid.....	12,436	10,000	13,666	18,000

A second experiment with a 450 unit plasma gave the following results:

Prep. 82.

Fractions.	A. 0.0-5.0	B. 0.0-3.3	C. 3.3-3.8	D. 3.8-5.0
Volume, c.c.	6,240	1,350	1,640	2,550
Units per c.c.....	1,050	900	1,300	1,600
Times concentrated	2.34	2.00	2.89	3.56
Per cent. recovered.....	72.8	13.9	22.6	45.3
Proteid, gms. per 100 c.c.....	10.59	12.06	13.46	13.41
Units, per gm. proteid.....	9,914	7,464	9,655	11,930

These observations show that the antitoxic globulins of the higher fractions are much more potent than those of the less soluble proteids.

Both the preparations, by the original method and by fractioning when precipitated from the NaCl solution and dialyzed, contained a probably partially denaturalized antitoxic globulin; this has a diminished solubility and antitoxic potency (per gm.) and is precipitated on slight acidification by diluting at least twenty times. The filtrates from the acid water precipitable globulin coagulated at 73 degrees while saline solutions of these precipitates showed varying and much lower coagulating temperatures. The following results were obtained on progressively fractioning (in two experiments) by the addition of the dry salt, to a liter of about 400 units antitoxic plasma. The initial dilution was 1.5. The precipitates were pressed between filters and extracted with saturated NaCl solution. The determinations on the filtered extracts are given per c.c. of the original plasma.

Fractioning of Plasma, 306, August 1, 1906.

Fractions.	Proteid per c. c.	Units per c. c.	Units per gm.
			Proteid.
A.			
0.0—3.4	0.00321	25	7,800
3.4—3.6	0.00223	20	8,960
3.6—3.8	0.00450	45	10,000
3.8—4.0	0.00416	52	12,480
4.0—4.2	0.00408	60	14,700
4.2—4.4	0.00272	50	18,390
4.4—4.6	0.00191	40	20,890
4.6—4.8	0.00163	30	18,410
4.8—5.0	0.00111	20	18,016
5.0—5.6	0.00428	15	6,175

Fractions.	Proteid per c. c.	Units per c. c.	Units per gm. Proteid.
B.			
0.0—3.4	0.00394	25	6,345
3.4—3.6	0.00219	20	9,114
3.6—3.8	0.00392	45	14,440
3.8—4.0	0.00335	52	15,530
4.0—4.2	0.00326	60	17,850
4.2—4.4	0.00255	55	21,600
4.4—4.6	0.00181	40	22,100
4.6—4.8	0.00147	30	20,380
4.8—5.0	0.00090	20	22,320
5.0—5.6	15

Further fractioning after complete removal of the water precipitable globulin was done on 50 c.c. of the globulin solution Prep. 77 A. The fractioning was with a dilution of the original preparation of 1:20. The results are expressed per c.c. of the original undiluted preparation.

Refractionation of Prep. 77 A.

Fractions.	Proteid per c. c.	Units per c. c.	Units per gm. Proteid.
0.0—4.0	0.0408	400	9,791
4.0—4.4	0.0165	225	13,667
4.4—5.0	0.0176	375	21,306
5.0 +	0.0018	75	41,722
4.8—5.5*	0.0046	150	34,783

* Made on a second 50 c.c. of the same preparation.

Prep. 82 D was refractioned without removing the acid-water precipitable globulin. The dilution was 1:10.

Refractionation of Prep. 82 D.

Fractions.	Proteid per c. c.	Units per c. c.	Units per gm. Proteid.
0.0—4.0	0.07318	600	8,136
4.0—4.2	0.01779	240	13,490
4.2—4.4	0.02197	260	11,840
4.4—4.6	0.01232	160	12,990
4.6—4.8	0.00708	90	12,711
4.8—5.0	0.00511	80	15,670
5.0—5.6	0.00197	90	45,690
	<hr/> 0.13941 <hr/>	<hr/> 1520 <hr/>	
For 82 D.	0.1341	1600	

This shows a globulin of rather uniform potency per gm. from fractions 4.0—4.8 and then a marked jump from 5.0—5.6 to about three times the original potency per gram. The proportion of antitoxin in the highest fractions is less than 6 per cent. of the total units. Prepared for administration as is the ordinary antitoxic globulin, the resulting product would have a potency of from 5000-6000 units per c.c.

From the data presented it appears that the saturated NaCl soluble serum globulins of the higher fractions of plasma are uniformly much more potent per gm. proteid than are those precipitated by lower concentrations of ammonium sulphate. Between concentrations of the sulphate of 5.0 and 5.6, a small proportion of the total NaCl soluble globulin preparation (or of a higher fraction of the same) is precipitated; the solution of this globulin has a protective power of from 40,000 to 45,000 units per gm. proteid.

Further investigations directed towards the purification and concentration of antitoxins are now in progress.

THE PRODUCTION OF DIPHTHERIA ANTITOXIN DURING THE YEAR 1906.

By Dr. EDWIN J. BANZHAF.

The amount of diphtheria antitoxic serum and citrated-plasma produced during the year 1906 was 1,680,685 c.c., of which 165,355 c.c. was serum and 1,515,330 c.c. citrated-plasma.

This amount was produced from 26 horses.

The average number of units per c.c. was 355; the highest number of units per c.c. being 1,450 and lowest 100.

By Gibson's method of concentrating and refining* we used 1,252 liters citrated-plasma averaging 400 units per c.c., and recovered 363½ liters, averaging 1,000 units per c.c.; the highest product thus produced was 2,500 units per c.c. and the lowest 400 units per c.c.

Horses, 305, 306, 307 and 308, which received toxin precipitated and concentrated by alcohol, are especially interesting, as can be seen from the following table. The first two, after only six weeks' immunization, each receiving in that time 330,750 m. l. d., produced an extremely potent antitoxic serum, each containing 1,200 units per c.c. After receiving an injection of 137,500 m. l. d. additional, and bleeding after a week, horse 305 tested 1,250 units per c.c. and horse 306 tested 1,450 units per c.c. Horse 305, receiving increasing amounts of toxin and bleeding each week, held between 1,200 and 1,250 units per c.c. for six bleedings, dropping to 1,000 units per c.c. and remaining there for three bleedings, and then slowly dropping until after 14 more bleedings the serum tested 300 units per c.c.

Horse 306, receiving each week increasing amounts of toxin, and bleeding each week, held between 1,300 and 1,450 units per c.c. for six bleedings, dropping to 1,100 units per c.c. and remaining there for four bleedings, and then slowly dropping until after 11 more bleedings the serum tested 400 units per c.c.

Horses 307 and 308, however, with exactly the same treatment, did very poorly.

* *Journal of Biological Chemistry*, i, 161, 1906, and *Studies from the Research Laboratory*, Vol. I., 1905.

Horse 307, after receiving as much as 1,108,750 in l. d., tested only 350 units per c.c. This horse, during the next three weeks, received 825,000 m. l. d. additional, testing then only 300 units per c.c. Although receiving increasing amounts of toxin each week and bleeding on the average of once a month, horse 307 continually dropped in potency and at last bleeding tested a little below 200 units per c.c.

Horse 308, after receiving 617,500 m. l. d., tested only 250 units per c.c. This horse, during the next four weeks, received 912,500 m. l. d. additional, testing then only 200 units per c.c. Although receiving increasing amounts of toxin each week and bleeding on the average of once a month, horse 305 continually dropped in potency and at last bleeding tested 100 units per c.c.

It is probable that even under the regular treatment horses 305 and 306 would have produced high antitoxic serum, and horses 307 and 308 low antitoxic serum.

We believe that the smaller amount of deleterious substances introduced in the injections with precipitated and concentrated toxin makes it possible to give the horses larger amounts of toxin without ill effects and to continue such injections without depressing the animal's vitality.

On the whole the use of precipitated and concentrated toxin does not seem at this time to offer any advantages which are not considerably outweighed by the disadvantages, viz.: expense of alcohol, loss of toxin, labor, etc.

Horse 262 shows the enormous capacity for antitoxic production which some animals possess. This horse entered the station in February, 1904, and soon began to produce a high-grade antitoxic serum. During the year 1904 he produced 81,010 c.c. serum, averaging 496 units per c.c.; the highest potency being 700 units per c.c. and the lowest 300 units. During the year 1905 he produced 69,585 c.c. serum, averaging 400 units per c.c., the highest being 550 units per c.c. and the lowest 250 units. During the year 1906 he produced 165,010 c.c. plasma and serum, averaging 409 units per c.c., the highest being 550 units per c.c. and the lowest 300 units, making the total production up to December 31, 1906, 315,605 c.c. plasma and serum, averaging 435 units per c.c.

Horse 299 is worthy of mention. Although a small horse, he produced, from February, 1906, to December, 1906, 180,290 c.c. plasma and serum, with an average of 462 units per c.c., the highest potency being 550 units per c.c. and the lowest 350 units per c.c.

Horse 262, during the year 1906, was bled 25 times, and horse 299 27 times, without any apparent ill effects, producing a fairly high grade antitoxic serum, with an average of 7 liters per bleeding.

One cannot but marvel at the splendid recuperative power of the animal organism.

TABLE I.

Number of Horse.	Date of First Injection.	Number of Bleedings during 1906.	Citrated Plasma produced in c.c. during 1906.	Serum produced in c.c. during 1906.	Total Plasma and Serum produced in c.c. during 1906.	Highest Potency in Units per c.c. during 1906.	Average Units per c.c. during 1906.	Final Disposition.
234.....	August 21, 1903	11	61,350	20,050	81,400	425	398	Bled to death April 24, 1906.
262.....	February 15, 1904	25	144,800	20,210	165,010	550	409	Still in use.
264.....	February 28, 1904	2	6,760	6,760	375	350	Died from injection February 12, 1906
268.....	April 18, 1904	2	11,190	11,190	300	275	Died, abscess in lungs February 20, 1906.
271.....	November 2, 1904	6	53,970	53,970	350	230	Bled to death, May 11, 1906.
272.....	November 2, 1904	6	29,000	29,000	350	230	Bled to death April 3, 1906.
284.....	July 13, 1905	3	12,400	12,400	200	200	Transferred to dispensary.
285.....	July 28, 1905	5	31,860	13,655	45,515	400	370	Died after bleeding March 8, 1906.
286.....	August 18, 1905	12	73,210	5,370	78,680	300	261	Died from colic May 3, 1906.
288.....	August 22, 1905	15	96,400	10,015	106,415	600	533	Died while being bled May 19, 1906.
289.....	August 22, 1905	6	21,590	6,480	28,070	200	200	Destroyed, autopsied December 26, 1906.
291.....	August 22, 1905	12	74,150	74,150	375	290	Bled to death May 16, 1906.
293.....	September 4, 1905	13	85,330	85,330	375	320	Bled to death May 16, 1906.
295.....	September 17, 1905	7	46,590	46,590	300	230	Bled to death April 6, 1906.
296.....	September 17, 1905	12	81,420	81,420	300	240	Bled to death November 28, 1906.
299.....	September 22, 1905	27	174,830	5,460	180,290	550	462	Destroyed, autopsied December 28, 1906.
302.....	November 8, 1905	4	32,300	32,300	200	200	Bled to death May 16, 1906.
*305.....	February 14, 1906	23	135,080	14,310	149,390	1,250	513	Died after injection December 21, 1906.
*306.....	February 14, 1906	21	120,600	18,410	139,010	1,450	587	Still in use.

* Horses were injected with concentrated and refined toxin.

TABLE I. (Continued.)

Number of Horse.	Date of First Injection.	Number of Bleedings during 1906.	Citrated Plasma produced in c. c. during 1906.	Serum produced in c. c. during 1906.	Total Plasma and Serum produced in c. c. during 1906.	Highest Potency in Units per c. c. during 1906.	Average Units per c. c. during 1906.	Final Disposition.
*307.....	February 14, 1906	7	36,600	2,145	38,745	350	230	Still in use.
*308.....	March 6, 1906	6	31,750	2,750	34,500	250	180	Bled to death August 28, 1906.
309.....	September 29, 1906	Test.	200	Still in use.
310.....	March 20, 1906	11	71,000	1,330	72,330	725	597	Bled to death August 16, 1906.
311.....	June 23, 1906	12	57,000	18,030	75,030	950	783	Died after injection December 21, 1906.
312.....	June 23, 1906	2	8,000	5,570	13,570	450	400	Died after injection October 20, 1906.
314.....	July 2, 1906	1	134,000	13,400	250	250	Destroyed, autopsied December 28, 1906.
316.....	5	22,700	3,620	26,320	250	495	Still in use.
317.....	November 13, 1906	Still in use.

* Horses were injected with concentrated and refined toxin.

TABLE II.

The total amount of plasma and serum produced by the older horses since entering the Station is as follows:

Number of Horse.	Serum Produced during 1903.	Average Units per c.c. during 1903.	Serum Produced during 1904.	Average Units per c.c. during 1904.	Serum produced during 1905.	Average Units per c.c. during 1905.	Plasma and Serum produced during 1906.	Average Units per c.c. during 1906.	Total Plasma and Serum produced in c.c. to December 31, 1906.	Average Units per c.c. to December 31, 1906.
234.....	30,775	475	67,265	472	60,150	375	81,400	398	239,590	430
262.....	81,010	496	69,585	400	165,010	409	315,605	435
264.....	59,305	563	40,760	382	6,760	350	106,865	432
268.....	30,325	525	36,500	440	11,190	275	78,015	413
271.....	42,710	310	53,970	230	96,680	270
272.....	35,615	312	29,000	230	64,615	271
284.....	14,190	290	12,400	200	26,590	245
285.....	24,535	412	45,515	370	70,050	391
286.....	5,010	250	78,680	261	83,690	255
288.....	5,250	400	106,415	533	111,665	466
289.....	3,740	250	28,070	200	31,810	275

THE RESISTANCE OF DIFFERENT STRAINS OF TYPHOID BACILLI IN CROTON TAP WATER.

By MARY E. GOODWIN, M. D., and ALICE ASSERSON, M. D.,
Assistant Bacteriologists.

Jordan,* Russell and Zeit tested the viability of typhoid bacilli in Lake Michigan water and in the water of the Chicago Drainage Canal. Russell† and Fuller, in their work on "The Longevity of the Typhoid Bacillus in Natural Waters and in Sewage," used Lake Mendota water and added sewage. Jordan, Russell and Zeit did fifteen experiments with Lake Michigan water in glass containers. The water contained from 100 to 200 saprophytes. In raw tap water the typhoid bacilli lived 6 to 7 days, in sterile tap water 25 days and in filtered tap water only 4 days. When the typhoid bacilli were put into raw tap water in parchment or celloidin sacs they lived 6 to 7 days. When the sacs were filled with sterile tap water the bacilli still survived at the end of 15 days when the experiments were discontinued. These 15 experiments seem to have been carried on with old cultures from different sources. The same workers did a good deal of work with Chicago river water when they used three recently isolated cultures. In this work the sacs were suspended in running water. In the Chicago river water the typhoid bacilli were isolated for only 3 days, and in the Drainage Canal water for only 2 days, with one exception, where it was found on the tenth day.

Russell and Fuller, in 1906, working with one of the same cultures, tested Lake Mendota water pure and with raw sewage added. In the flowing water the typhoid bacilli lived 8 to 10 days; with the addition of sewage only 3 to 5 days.

The present work was undertaken to find out the effects of Croton tap water on different strains of typhoid bacilli exposed in different ways. The following cultures were used:

Six cultures freshly isolated from the stools of two typhoid cases, in the second week, Stone 1, 3 and 5, and Ruth 1, 2 and 5; two cultures

* *Journal of Infectious Diseases*, 1904, i, p. 641.

† *Journal of Infectious Diseases*, Supplement No. 2, 1906.

isolated six months previously, 183 and 167; Mt. Sinai and NYH₃ isolated 2 years. Pf. isolated 14 years.

Where raw water was used the typhoid bacilli were isolated from Conradi-Drigalski plates and identified by specific sera and by sugar media.

The first experiment included tests of sterile distilled, sterile tap and filtered tap water with the six freshly isolated cultures and raw tap water with the same cultures and Mt. S., NYH₃, 183 and 167.

Table I.

The three cultures from the Stone case all died on the fourth day in sterilized distilled water, and on the thirtieth day in sterilized tap water. In filtered tap water two cultures lived 9 days longer than the third. In sterile distilled water the cultures from Ruth case seemed much more resistant than those from the Stone case, as they lived 6, 17 and 18 days. In filtered tap water the relation seemed reversed, the Ruth cultures living only 21 days, while two of the Stone cultures lived 30 days.

In raw tap water, with 470 colonies developing from 1 cc. at 37° and 6,480 at 24°, one of the freshly isolated cultures, Ruth 5, lived only 5 days, while all the old laboratory cultures lived more than 11 days.

In the work with different kinds of sacs filled with sterile tap water the technique was about the same as that used by Russell and Fuller. Gelatin sacs made by covering silk with 40 per cent. gelatin and hardening in 40 per cent. formalin were used instead of agar on fiber sacs. These, after hardening, could be sterilized in the Arnold sterilizer without injury. Heavy parchment paper was used instead of the parchment tubing, as the only tubing obtainable in New York was found to contain so many small holes that it was unfit for use.

TABLE I.

Culture.	Number of Bacilli put into the c.c.	Last Day of Isolation.
Sterile distilled water in glass receptacles—		
Stone 1.....	214,000	4th
“ 3.....	179,000	4th
“ 5.....	111,000	4th
Ruth 1.....	153,000	6th
“ 2.....	88,000	18th
“ 5.....	257,000	17th
Sterile tap water in glass receptacles—		
Stone 1.....	214,000	30th
“ 3.....	179,000	30th
“ 5.....	111,000	30th
Ruth 1.....	153,000	30th
“ 2.....	88,000	12th
“ 5.....	257,000	30th
Filtered tap water in glass receptacles—		
Stone 1.....	214,000	21st
“ 3.....	179,000	30th
“ 5.....	111,000	30th
Ruth 1.....	153,000	21st
“ 2.....	88,000	21st
“ 5.....	257,000	21st
Raw tap water in glass receptacles—		
Stone 1.....	214,000	12th
“ 3.....	179,000	17th
“ 5.....	111,000	17th
Ruth 1.....	153,000	13th
“ 2.....	88,000	21st
“ 5.....	257,000	5th
Mt. S. *.....	100,000	17th
N. Y. H. _s *.....	100,000	17th
183 *.....	100,000	17th
167 *.....	100,000	12th

* Count estimated, the plates having so many colonies that they were uncountable.

TABLE II.

Parchment and Celloidin Filled with Sterile Tap Water and Placed in Running Tap Water.

Culture.	Parchment Sacs, Number of Bacilli put in per c.c.	Last Day Isolated.	Celloidin Sacs, Number of Bacilli Put in per c.c.	Last Day Isolated.
First Set of Experiments.				
Stone 1.	32,000	8th	75,000	8th
Stone 3.	129,000	8th	86,000	7th
Stone 5.	194,000	7th	54,000	6th
Ruth 1.	65,000	8th	86,000	6th
Ruth 2.	54,000	7th	10,800	6th
Ruth 5.	108,000	7th	66,400	4th
Mt. S.	10,800	4th
Pf.	64,800	4th
Second Set of Experiments.				
Stone 1.	300,000	5th	300,000	21st
Stone 3.	285,000	7th	285,000	11th
Stone 5.	200,000	6th	200,000	4th
Ruth 1.	600,000	5th	600,000	21st
Ruth 2.	121,000	5th	121,000	4th
Ruth 5.	675,000	7th	675,000	20th
Mt. S.	531,000	7th	531,000	9th
Pf.	121,000	5th	121,000	11th

GELATIN SACS.

Culture.	Number of Bacilli to c.c.	Last Day Isolated.
Experiment 1.		
Mt. S.	13,500	8th
Stone 1.	24,000	8th
Stone 3.	20,000	8th
Ruth 5.	20,000	8th
Experiment 2.		
Mt. S.	300,000	8th
Pf.	300,000	8th
183.	300,000	8th

TABLE III.

The Last Day of Isolation of the Different Typhoid Cultures in the Different Sacs in Running Tap Water.

Culture.	Parchment.	Celloidin.	Gelatin.
Stone 1.....	8th day	21st day	8th day
Stone 3.....	8th day	11th day	8th day
Stone 5.....	7th day	6th day
Ruth 1.....	8th day	21st day
Ruth 2.....	7th day	6th day
Ruth 5.....	7th day	20th day	8th day
Mt. S.....	7th day	9th day	8th day
Pf.....	5th day	11th day	8th day
183.....	8th day

Conclusions.

All the cultures except the one which had been grown on artificial media for 14 years, seemed to have about the same resistance in running tap water. The long cultivated strain generally died out a day or two earlier than the other cultures.

The resistance of typhoid bacilli in Croton tap water corresponds more nearly to the resistance in comparatively pure water such as that of Lake Mendota than to that of a polluted water such as the Chicago river water.

REPORT OF BACTERIOLOGICAL EXAMINATION OF CROTON WATER FOR THE YEAR 1906.

By MARY E. GOODWIN, M. D., Bacteriologist.

Croton tap water at East Sixteenth street was plated in agar, and also tested for the presence of colon bacilli, once a week during the year.

Date.	1 c.c. Plated in Agar at 37° C. for 24 Hours.	At 24° C. for 72 Hours.	Smallest Quantity of Water con- taining Colon Bacilli as shown by the Presumptive Test.	Gas Production.	
	Number of Colonies.			Per Cent. Gas.	Proportion.
January 3.....	250	3,000	1 c.c.	30	{ 25 per cent. CO ₂ H present.
January 11.....	76	141	10 c.c.	* Few bubbles
January 22.....	200	4,800	10 c.c.	30	{ 25 per cent. CO ₂ H present.
January 30.....	27	46	1 c.c.	90	{ 35 per cent. CO ₂ H present.
February 7*.....	22	848	1/10 c.c.	25	{ 25 per cent. CO ₂ H present.
February 7†.....	166	2,640	1/500 c.c.	60	{ 40 per cent. CO ₂ H present.
February 7 §.....	20,000	40,000	1/100 c.c.	20	{ 25 per cent. CO ₂ H present.
February 7 	44	2,160	1 c.c.	2
February 8.....	880	2,048	10 c.c.	20	{ 25 per cent. CO ₂ H present.
February 15.....	46	628	No gas in 10 c.c.
February 22.....	60	720	10 c.c.	15	{ 5 per cent. CO ₂ H present.
March 2.....	144	6,000	1/10 c.c.	90	{ 60 per cent. CO ₂ H present.
March 10.....	290	3,140	1/10 c.c.	5
March 17.....	50	240	5 c.c.	35	{ 30 per cent. CO ₂ H present.
March 23.....	96	1,140	10 c.c.	65	{ 25 per cent. CO ₂ H present.
March 29.....	1,270	2,250	10 c.c.	3
April 13.....	80	1,920	10 c.c.	50	{ 20 per cent. CO ₂ H present.
April 18.....	46	260	10 c.c.	1
April 26.....	55	1,680	1 c.c.	30	{ 30 per cent. CO ₂ H present.
May 3.....	37	500	10 c.c.	* Few bubbles
May 9.....	480	810	10 c.c.	40	{ 25 per cent. CO ₂ H present.
May 17.....	11	130	10 c.c.	40	{ 30 per cent. CO ₂ H present.
May 24.....	22	114	10 c.c.	* Few bubbles
May 31.....	22	10 c.c.	25	{ 25 per cent. CO ₂ H present.
June 10.....	220	3,500	1/10 c.c.	20	{ 20 per cent. CO ₂ H present.
June 14.....	28	530	1/10 c.c.	25	{ Apparently no CO ₂ H present.
June 22.....	540	2,380 {	1/10 c.c. 1 c.c.	10 25	{ 25 per cent. CO ₂ H present.

* These bubbles of gas were produced by the bacterial growth and probably indicate colon bacilli.

Date.	1 c.c. Plated in Agar at 37° C. for 24 Hours.	At 24° C. for 72 Hours.	Smallest Quantity of Water con- taining Colon Bacilli as shown by the Presumptive Test.	Gas Production.	
	Number of Colonies.			Per Cent. Gas.	Proportion.
June 28.....	147	260	1/10 c.c.	30	30 per cent. CO ₂ H present.
July 5.....	39	179	1/10 c.c.	10	20 per cent. CO ₂ H present.
July 12.....	36	103	1/10 c.c.	25	40 per cent. CO ₂ H present.
July 19.....	310	340	1/10 c.c.	35	20 per cent. CO ₂ H present.
July 26.....	80	170	1/10 c.c.	25	30 per cent. CO ₂ H present.
August 1.....	380	1,170	1/10 c.c.	40	30 per cent. CO ₂ H present.
August 10.....	220	430	1/10 c.c.	12	30 per cent. CO ₂ H present.
August 15.....	50	160	1 c.c.	20	25 per cent. CO ₂ H present.
August 22.....	400	368	1/10 c.c.	25	50 per cent. CO ₂ H present.
August 30	237	700	1/100 c.c.	20	30 per cent. CO ₂ H present.
September 6.....	400	380	1/10 c.c.	12	25 per cent. CO ₂ H present.
September 15.....	110	530	1/10 c.c.	8	25 per cent. CO ₂ H present.
September 20.....	44,032	440	1 c.c.	90	50 per cent. CO ₂ H present.
September 24.....	45	780	1 c.c.	95	70 per cent. CO ₂ H present.
September 28.....	135	860	1/10 c.c.	20	25 per cent. CO ₂ H present.
October 3.....	18	237	1/10 c.c.	25	33½ per cent. CO ₂ H present.
October 12.....	25	157	1/10 c.c.	16	60 per cent. CO ₂ H present.
October 19.....	2,400	3,000	1/10 c.c.	95	10 per cent. CO ₂ H present.
October 24.....	560	2,100 {	1/10 c.c. 1 c.c.	10 80	40 per cent. CO ₂ H present.
November 1.....	49	440	1/10 c.c.	10	25 per cent. CO ₂ H present.
November 14.....	41	169	1/10 c.c.	30	25 per cent. CO ₂ H present.
November 21.....	18	141	10 c.c.	60	50 per cent. CO ₂ H present.
November 28.....	43	93	10 c.c.	90	50 per cent. CO ₂ H present.
December 5.....	22	320	10 c.c.	90	40 per cent. CO ₂ H present.
December 12.....	52	270	1 c.c.	20	25 per cent. CO ₂ H present.
December 20.....	137	450	1/10 c.c.	30	25 per cent. CO ₂ H present.

NOTE—* Specimen from Kisco Brook above drain.

† Specimen from Kisco Hotel drain.

§ Specimen from Gorham Pond Brook.

|| Specimen from Outlet from Croton Lake.

A STUDY OF THE PNEUMOCOCCUS DURING LONG CONTINUED CULTIVATION ON MEDIA, WITH ESPECIAL REFERENCE TO THE INULIN TEST.*

JANE L. BERRY, M. D.

The work reported in this paper was carried on under the direction of Drs. William H. Park and A. W. Williams, and consists of a study of some of the pneumococcus strains isolated at the Research Laboratory during the period between November, 1904, and August, 1905. The system of designating the strains is that used by Drs. Park and Williams in the report made to the Pneumonia Commission,^a the description of the strains here mentioned being included in that report.

The organisms have been cultivated during the time since isolation upon blood agar, with occasional transfers to serum broth, or calcium broth. The possibility of contamination by similar organisms, during so long a period of cultivation on artificial media, has, of course, to be considered in judging results, but it is not thought that any contamination has taken place; first, because of the care exercised in transferring cultures and watching results; second, because where changes have occurred in the different strains they have been of a similar character, indicating a systematic tendency; and third, because in some cases, where a change has taken place, it has been possible to observe the different stages leading toward it, in a graduated series.

Very few strains are now found to be typical, morphologically. The majority show very small organisms and increased chain formation, namely, a more or less marked approach toward a streptococcus type. With some strains this change seems to be a permanent one, the organisms having a definite streptococcus morphology in all media; with others the change is but temporary, to be followed in later cultures by a return to a characteristic pneumococcus type, and with a few strains, while an occasional atypical culture is seen, the majority of cultures

* This is a continuation of the work reported in the 1905 volume of "Studies from the Research Laboratory."

a Jour. Exper. Med., 1905, 7, p. 403.

now show a morphology as typical as when the organisms were first isolated more than a year ago.

Table I gives the results of a study of morphology and inulin coagulation made during January, 1906. Sixty-one strains were studied at this time, selected at random from the laboratory stock. Since but one inulin test was made with each strain in this series of experiments, only general conclusions can be drawn as to inulin coagulation from this part of the work, in view of the great irregularities later observed.

TABLE I.

Summary of Inulin Tests Made January, 1906, upon Strains of Pneumococci Isolated at Different Periods between November, 1904, and March, 1905, Compared with Similar Tests Made Soon after Isolation. All Positive in Original Tests.

	Coagulated not Later Than in Original Test		Coagulated Later than in Original Test		Not Coagulated This Time Though Coagulated in Original Test.		Total
	Char.	Not Char.	Characteristic	Not Char.	Char.	Not Char.	
Pneumonia Cases	8 ₁₀₀₁	18 ₁₁₂₀	15 ₁₁₁₂ 3d later	22 ₁₀₀₁ 2d later	4 ₁₀₀₁	20 ₁₁₀₀	27
	9 ₁₀₀₁	77 ₁₀₀₁	39 ₁₀₀₁ 2d "			21 ₁₁₀₀	
	13 ₁₀₀₁		47 ₁₁₂₀ 2d "		62 ₁₁₁₂	36 ₁₀₀₁	
	46 ₁₀₀₁		57 ₁₁₂₀ 8d "		67 ₁₀₀₁	*47 ₁₀₀₁	
	56 ₁₁₁₂		83 ₁₀₀₁ 10d "		72 ₁₀₀₁		
	69 ₁₀₀₁				75 ₁₀₀₁	73 ₁₀₀₁	
	82 ₁₂₀₂				76 ₁₀₀₁		
					98 ₁₁₂₂		
Normal Cases	7	2	5	1	7	5	23
	9		6		12		
	N41 ₁₁₀₀	N106 ₁₁₁₂	N89 ₁₁₀₀ 8d later		N23 ₁₁₀₀	*N10 ₁₁₁₂	
	N124 ₁₀₀₁	N112 ₁₀₀₁			N51 ₁₁₀₀	N11 ₁₁₀₀	
Miscellaneous Cases	N135 ₁₁₀₀	N127 ₁₁₁₂	N99 ₁₁₀₀ 9d later		N52 ₁₁₀₀	N43 ₁₁₂₀	11
	N36 ₁₂₁₀	N113 ₁₀₀₁			N59 ₁₁₀₀	N45 ₁₀₀₁	
					N97 ₁₂₀₂	N107 ₁₁₁₂	
					N100 ₁₁₀₀		
Miscellaneous Cases	4	4	2	0	8	5	13
	8		2		13		
	C&D4 ₁₀₀₁	C23 ₁₁₁₂	C5 ₁₁₁₂ 1d later	Me2 ₁₁₁₂ 3d later	C&D8 ₁₀₀₁	*C4 ₁₁₀₀	
	Q5 ₁₁₀₀	Me1 ₁₁₀₀			T3 ₁₁₀₀		
Miscellaneous Cases	2	2	1	1	Q3 ₁₀₀₁		5
	4		2		C23 ₁₁₂₀		
Total.....							61

* *Pneumococcus mucosus*.

Twenty-seven strains were studied which came originally from cases of pneumonia. Nine of these coagulated inulin not later than in original tests. Seven were fairly characteristic morphologically, two not characteristic; six coagulated later than in original tests, five characteristic, one not characteristic; while 12 did not coagulate in these cultures, though all gave a positive reaction when first tested. When first studied at the time of isolation, 21 of the above were typical, six fairly typical.

Twenty-three strains from normal cases were studied, eight of these coagulating inulin not later than in first tests, four being characteristic, four not characteristic; two coagulated later than in first tests, both characteristic, and 13 did not coagulate at this time, although positive in original tests. When first studied 13 were typical, four not characteristic, and six atypical.

Of the 11 strains from miscellaneous cases, four coagulated inulin, not later than when first studied, two characteristic, one not characteristic; and five failed to coagulate, although positive in original tests, four of these characteristic, one not characteristic. When first studied six were typical, five not characteristic.

Unless extremely small, size of organisms has not been considered in this division into characteristic and non-characteristic, the classification being based upon the general morphological picture.

In order to ascertain the effect upon the inulin fermenting property produced by passage of the organism through animals, a certain number of strains were selected for further study, and in March, and again in June, of the present year, these were inoculated into mice and rabbits.

In these experiments no attempt was made to ascertain the smallest lethal dose, but all strains were found to have lost virulence, very large doses being required to kill the animals. In the last series of mouse inoculations (Mouse 1, Mouse 2, etc.) the organism was passed directly from one animal to another; the heart being divided, the chest cavity then washed with sterile water, and this inoculated intraperitoneally, cultures being made at the same time. By this method, a high degree of virulence was developed in some cases, mice dying within six to eight hours after inoculation, with profuse cultures of the pneumococcus given by the heart's blood. See Table 2.

TABLE 2.

Synopsis of Characteristics of Pneumococcus Cultures before Passage through Animals, and after Passage through Last Series.

Original Culture				Original at time of Isolation	Original January, 1906	Original before Inoculation May, 1906	Mice					Original June, 1906
							1	2	3	4	5	
41001	Morphology	Typ.		+	±	±	±		+		+
		Atyp.		Dis- carded	Lived
		Caps.		+	-		+	
		Inulin		+	-	-	-		+		-
†161112	Morphology	Typ.		±	±+	+	±
		Atyp.		+	+	±+	Dis- carded	+
		Caps.		?	?	-	-		Indic.
		Inulin		+	-	-	±	±	±		+	+
361001 (No. 1)	Morphology	Typ.		+
		Atyp.		+	+	Dis- carded	+	+	+	Lived	+
		Caps.		+		-	-	No org	
		Inulin		+	-	-		-	-	-		-
361001 (No. 2)	Morphology	Typ.		+	+	+		+
		Atyp.		Not inoc.
		Caps.		+
		Inulin		+	+	+		+
471001	Morphology	Typ.		+	+	±+	±+	+	±+	+	+	+
		Atyp.	
		Caps.		+	-	-	-	-	No org
		Inulin		+	-	-	±	-	±	±	-	+
761001	Morphology	Typ.		+	±
		Atyp.		+	+	+	+	+	+	+
		Caps.		Indic.	Indic.	-	-	-	-
		Inulin		+	-	-	+	±	-	±	±	-
N231122	Morphology	Typ.		+	±	+	+	+	±+	±	+	+
		Atyp.	
		Caps.		+	?	+	-	+	+
		Inulin		+	-	-	+	+	±	±	+	+
N911222	Morphology	Typ.		+	±	+	+	+	+		+
		Atyp.		Lived
		Caps.		+	+	+	+		+
		Inulin		+	-	-	+	+	+		+

*+±=Plate typical, serum broth not characteristic.
†±+=Plate not characteristic, serum broth typical.
1001=Organism isolated from plate.

1122=Organism isolated from 1st rabbit.
1222=" " " 2d " "
1112=" " " mouse.

TABLE 3.
Total Inulin Tests: 4 Cases.

Case	Strain		Date of Culture					Total Results			Total Tests
			At Time of Isolation	January, 1906	March, 1906	May, 1906	June, 1906	-	±	+	
N23,1122.....	Original strain	-	..	1	2	2	3	8	11
		±	1	..	1	..	
		+	1	1	2	
	M. A	-	2	2	2
		±	1	1	..	2	
		+	1	1	..	
	R. A.....	-	1	1	..	2	4
		±	1	1	..	
		+	1	1	
	R. B.....	-	1	3	4	9
		±	1	..	1	..	
		+	1	..	3	4	
	M1.....	-	6
		±	3	..	3	..	
		+	3	3	
	M2.....	-	7
		±	4	..	4	..	
		+	3	3	
	M3.....	-	1	1	3
		±	2	..	2	..	
		+	
	M4.....	-	1	1	3
		±	2	..	2	..	
		+	
	M5.....	-	1	1	5
		±	2	..	2	..	
		+	2	2	
	M. R. 1	-	4	4	9
		±	3	..	3	..	
		+	2	2	
	M. R. 2.....	-	4
		±	3	..	3	..	
		+	1	1	
Total.....								23	22	18	63

TABLE 3.—Continued.

Case	Strain		Date of Culture					Total Results			Total Tests.	
			At Time of Isolation	January, 1906	March, 1906	May, 1906	June, 1906	—	±	+		
No 1, 1922.....	Original strain.....	—	..	1	2	3	6	12	15	
		±		
		+	3	3		
		M. A.....	+	1	1	1
		M. B.....	+	2	2	2
		M. C.....	—	1	..	1	8
			±	1	..	1	..	2	..	
			+	1	1	3	5	
		R. A.....	+	2	2	
		R. B.....	—	5
			±	1	..	1	..	
			+	1	1	2	4	
		M ₁	—	2	2	2
			±	
			+	
		M ₂	—	3	3	3
			±	
			+	
		M ₃	—	2	2	8
			±	2	..	2	..	
			+	4	4	
		M. M. 1.....	—	8
			±	
			+	8	8	
		M. M. 2.....	—	1	1	7
			±	2	..	2	..	
			+	4	4	
		M. R. 1.....	—	4
			±	
			+	4	4	
		M. R. 2.....	—	5
			±	1	..	1	..	
			+	4	4	
Total.....									21	8	41	

TABLE 3.—Continued.

Case	Strain		Date of Culture					Total Results			Total Tests		
			At Time of Isolation	January, 1906	March, 1906	May, 1906	June, 1906	—	±	+			
41001.....	Original strain.....	{	—	..	1	2	3	6	12	{	13
			±		
			+	1	1		
	M. A.....		—	2	2		2
	M1.....	{	—	2	2	4	{	4
			±		
			+		
	M3.....	{	—	{	4
			±	1	1	..	2	..		
			+	1	1	2		
	Total.....								18	2	3	23	

TABLE 3.—Continued.

Case	Strain	Date of Culture	Morphology			Inulin					Total Tests
			Typical	Atypical	Capsule	Total Results					
						-	Sl.	±	+	++	
N62 ₁₁₂₂	Original	Mar., '05	..	+	-	4	4
	"	Jan., '06	±	1	1
	"	Mar., '06	..	+	1	1
	*R. A.....	" '06	±	..	No org.	1	..	1
	R. B.....	" '06	..	+	No org.	1	1
	M. A.....	" '06	..	+	+	1	1
	Original	May, '06	..	+	1	1
	"	June, '06	..	+	3	1	2	2	..	8
	R. A.....	" '06	..	+	2	..	2	1	..	5
	†M. R. 1.....	" '06	±	..	-	1	1	10	2	..	14
	M. R. 2.....	" '06	..	+	No org.	2	..	3	1	..	6
	M. R. 3.....	" '06	±	..	-	1	..	2	1	..	4
	M. R. 4.....	" '06	..	+	Indic.	1	..	3	1	..	5
	R. R.....	" '06	..	+	-	3	1	4
Total.....						19	2	22	12	1	56

NOTE.

Total coagulating cultures in black-face figures.

M. A.=first mouse inoculated.

M. 1.= " " " of second series.

M. M. 1.=first mouse inoculated from mouse of first series.

M. R. 1.= " " " from rabbit.

R. A.=first rabbit inoculated.

R. B.=rabbit inoculated from rabbit.

*Plate atypical; serum broth typical.

†Plate typical; serum broth atypical.

Table 3 gives the complete record of inulin tests made with four strains, the results in these cases being typical of those given by the other cases studied.

Of the comparatively small number of cultures studied only two have coagulated the inulin medium in recent tests which failed to coagulate it when first isolated. These two, 16₁₁₁₂ and N 62₁₁₂₂, were not included in the January table.

No. 4₁₀₀₁ originally a large, typical pneumococcus, showing capsule and coagulating inulin, has in these tests shown a variable morpholog-

with loss of capsules and inulin-fermenting power, but after passage through Mouse 3 there is again a typical morphology, with capsules, and two cultures showing complete and two partial inulin coagulation. Of a total of 23 inulin tests, three are positive, two partial, and 18 negative. To this list may be added at least six coagulations not recorded, made during the first few months after isolation, when the organism was frequently tested and always gave prompt positive results. The negative tests have all been made since the beginning of January.

No. 16₁₁₁₂ which has shown a mixed streptococcus and pneumococcus morphology throughout cultivation, and was negative to inulin when first tested, now gives positive inulin coagulation in cultures, both from the original strain and after passage through Mouse 5. Of the total inulin tests three are positive, eight partial, and 20 negative.

No. 36₁₀₀₁ has been of special interest. It was originally a very typical pneumococcus, showing capsules, coagulating inulin promptly, and very virulent for animals. It now shows in two series of cultures from the same strain, entirely opposite characteristics. The one series consists of later transfers from a series which in the summer of 1905 was carried on for many generations on special media, namely: horse blood agar, rat blood agar, rabbit blood agar, and mouse blood agar; cultures being then tested for virulence and inulin coagulation. Originally virulent for mice in doses of 1/1,000,000 c.c., rabbits 1/5,000, and rats 1/10 c.c., all cultures were now found to have decidedly lost in pathogenic power for these animals. Inulin was still promptly coagulated in seven cultures. Tried again in October of the same year, the cultures were found to be non-virulent for rabbits in 4 c.c. doses, and no longer killed mice unless given in large doses of a strong emulsion. No further tests of virulence have been made with this series. The cultures are still fairly typical morphologically, have recently coagulated inulin, and have given 12 positive coagulations during the past six months.

The contrasting series of 36₁₀₀₁ consists of the regular laboratory stock cultures. In these a complete change has taken place, and the organism now grows as a typical streptococcus in all media, is cultivated with difficulty, and shows no reaction with inulin serum water. This change has been a gradual one, all degrees of morphology having

been observed between the two types of organisms, and repeated fishing from atypical colonies have given cultures of a similar mixed growth. Of 56 total inulin tests, 23 have been positive, 33 negative.

No. 47₁₀₀₁ is of peculiar interest on account of its morphological changes. Originally a typical *Pneumococcus mucosus*, it has shown in subsequent cultures a varying morphology, sometimes of a typical pneumococcus and again of a streptococcus type, with occasional reversions to the original *Pneumococcus mucosus*, the latter form generally appearing only after passage through animals, especially mice, or when a fresh transfer is made after a long resting period. After persisting for a varying number of transfers (from one or two to eight) the *Pneumococcus mucosus* form is again lost, the organism reverting to the pneumococcus, the streptococcus, or to mixed types. Table 4 shows the variations observed after continued cultivation on various media, according to plan described under 36₁₀₀₁. In the transfers following those given in the table all the horse blood agar cultures showed the *Pneumococcus mucosus* form, which persisted for eight weekly transfers, the pneumococcus, the streptococcus, or the mixed forms then reappearing. In January last a typical *Pneumococcus mucosus* was again obtained from this series, the form persisting for several transfers.

The changes of form observed in this organism are in unison with the results previously obtained at this laboratory, and are a confirmation of the intimate connection and interchangeable nature of the pneumococcus and *Pneumococcus mucosus* types of organism. (See Table 4.)

No. 47₁₀₀₁ was originally virulent for mice in 1/100,000 c.c., rats 1/100 c.c. Tested after growth on the various blood agars, a loss of virulence was found in all cultures. Some difference was noticed in cultures according to the media upon which growth had been carried on, but upon the whole the results were irregular, and without further tests no definite conclusion could be drawn upon this point.

The coagulation of inulin, while at first prompt, has since been irregular with this organism. Of 54 inulin tests, 13 are positive, 6 partial, and 35 negative.

No. 76₁₀₀₁ is another organism which has shown a marked change. Originally a typical pneumococcus, coagulating inulin promptly, in

these tests it has appeared as a characteristic short streptococcus, and with the exception of two positive coagulations from Mouse 1, and four partial reactions from other animals, all recent inulin tests have been negative. Of 31 total inulin tests three are positive, four partial, and 24 negative.

N 62₁₁₂₂ has shown features of peculiar interest and in the summary results have been given more in detail. With the exception of a few cultures which more nearly approach the pneumococcus type, this organism has presented the appearance of a typical streptococcus during the entire period of cultivation. It was negative to inulin when first isolated and in many subsequent tests, but in the recent series of experiments coagulations have been obtained with cultures from each animal, and also from the original strain. Of 56 inulin tests, 13 have been positive, 24 partial, and 19 negative.

N 23₁₁₂₂ and N 91₁₂₂₂ were both originally typical pneumococci, showing capsules, coagulating inulin promptly, and virulent for animals. With the exception of an occasional culture showing increased chain formation, both strains have been typical in morphology throughout, N 91₁₂₂₂ especially showing the most typical large pneumococci, but the record of inulin tests shows that with both strains many cultures have failed to coagulate inulin. After passage through animals coagulation again occurs, and recently positive inulin tests have also been obtained from the original strain of N 23. N 23₁₁₂₂ in 63 inulin tests shows 18 positive, 22 partial, and 23 negative; N 91₁₂₂₂ in a total of 70 tests gives 41 positive, eight partial, and 21 negative; we have therefore but 35 per cent. and 58.5 per cent. respectively of positive inulin tests for these two very typical strains of pneumococci.

TABLE 4.

Showing Variations in Morphology (Pneumococcus and Pneumococcus mucosus Forms), and Inulin Coagulation of 47_{mm} in August, 1906, After Continual Cultivation on Special Media. First Series Transferred Daily; Second Series After Interval of Nine Days.

Series.	Date of Culture.	Medium.	Total Transfer No.	Transfer on Special Medium.	Typ. Pneumococcus mucosus.		Typ. Pneumococcus.		Inulin Coagulation.
					Growth.	Smear.	Growth.	Smear.	
Horse Blood Agar	Transferred daily . . .	8/9 Horse bl. agar.	90	30	±	±			
		8/9 Serum broth..	90	+			
		8/10 Inulin.....	+	±
		8/11 Bl. agar plate.	±	±	
	Transferred 9th day.	8/9 Horse bl. agar.	85	25	+	+			
		8/9 Serum broth..	85	+	
		8/10 Inulin.....	+	+
		8/11 Bl. agar plate.	+	+			
Rabbit Blood Agar	Transferred daily....	8/9 Rabbit bl. agar	101	41	±	±			
		8/9 Serum broth..	101	±			
		8/10 Inulin.....	±	-
		8/11 Bl. agar plate.	+	+	
	Transferred 9th day .	8/9 Rabbit bl. agar	96	35	±	±			
		8/9 Serum broth..	96	±			
		8/10 Inulin	±	+
		8/11 Bl. agar plate.	±	±	
Rat Blood Agar.	Transferred daily....	8/9 Rat bl. agar ..	101	40	±	±			
		8/9 Serum broth..	101	±			
		8/10 Inulin.....	±	+
		8/11 Bl. agar plate.	+	+	
	Transferred 9th day .	8/9 Rat bl. agar ..	96	35	+	+			
		8/9 Serum broth..	96	±			
		8/10 Inulin.....	±	+
		8/11 Bl. agar plate.	±	±	

TABLE 4 (Continued.)

Series.	Date of Culture.	Medium.	Total Transfer No.	Transfer on Special Medium.	Typ. Pneumococcus mucosus.		Typ. Pneumococcus.		Inulin Coagulation.
					Growth.	Smear.	Growth.	Smear.	
Mouse Blood Agar	Transferred daily....	8/9 Mouse bl. agar	102	11	+	+			
		8/9 Serum broth..	102	+			
		8/10 Inulin.....	+	+
		8/11 Bl. agar plate.	+	+			
	Transferred 9th day.	8/9 Mouse bl. agar	97	6	+	+			
		8/9 Serum broth..	97	+			
		8/10 Inulin.....	+	+
		8/11 Bl. agar plate.	+	+	

Blood agar and serum broth cultures made from blood agar of August 8, in each case. Plates made from inulin cultures.

Considered as a whole, it will be seen that the entire series of inulin tests here reported is characterized by a marked irregularity as to coagulation results, this being the case not only with atypical, but also with typical strains.

As the tables show, passage through animals seems generally to have a favorable influence upon the inulin fermenting power of the pneumococcus, an effect best shown in the strain from N^o 91.

The number of organisms inoculated into the inulin medium is often found to be an important factor, as coagulations have several times been obtained by the use of a strong emulsion of organisms after many negative results with cultures of ordinarily abundant growth. This, however, is not an invariable rule, as good coagulations have been obtained in some cases from cultures showing a very poor growth, while other cultures of the same inulin lot, containing a heavy growth of the same organism, have failed to coagulate.

The growth of the organisms in inulin has been studied in all cases, either in smears or by blood-agar streak plates, whenever possible, by both methods, and no cultures have been included in the report which have not been found to contain an abundant number of organisms; the

large number discarded on account of insufficient growth causing the irregularity in the number of tests recorded for each strain. All inulin cultures were incubated for two weeks before being classed as negative.

The inulin serum water used in these tests was made according to the usual method, with one-third ox serum, two-thirds distilled water, and 1 per cent. inulin powder, each lot being tested with laboratory stock cultures before being used in these experiments. The inulin powder used in some of the earlier work was extracted by Dr. Gibson at the Research Laboratory from dandelion roots, a preparation which gave very good results. For all the other tests the medium used was prepared from Merck's inulin (white). Various stocks of this powder, procured at different periods were found to differ greatly, and one whole stock had to be discarded, as no reliable tests could be obtained from it. In all of the work done in March and subsequently but one stock of Merck's white inulin has been employed, 5 c.c. of the inulin serum water being inoculated with $\frac{1}{2}$ c.c. of culture in each test. The greatest variation has been found between lots of inulin serum water made at different dates from this one inulin powder, and from the same or different lots of ox serum. Tubes inoculated with the same strain show all stages between entirely negative and positive results and an equal irregularity is found in many cases when tubes of the same or different lots of inulin medium are inoculated simultaneously with the contents of the same culture tube. Some difference was noticed between lots of inulin medium according to the supply of ox serum used, but at the end of the time-limit differences based upon this distinction were either very slight or no longer noticeable. Table 5 gives the results of the tests made with N 23₁₁₂₂ and N 91₁₂₂₂ in five lots of inulin medium made of the same inulin powder, and two different lots of ox serum.

TABLE 5.

Results of Tests with Five Lots of Inulin Serum Water. All Made from Same Inulin Powder (Merck's White) from Two Lots of Ox Serum—2 Cases.

Case.	Strain.	Tests Grouped under Lot of Inulin Medium Used.											Total Tests.
		Tubes Inoculated.						Results.					
		1st Ox. Ser.			2d Ox. Ser.			Coagulation.					
		Lot.			Lot.			-	SL	+	+	++	
		1	2	3	4	5		-	SL	+	+	++	
N23 ₁₁₂₂	Orig.....	I	I	5
		..	I	I	
		I	I	
		I	I	..	
		I	I	
	M 1.....	I	I	6
		I	I	..	
		3	2	I	..	
		I	I	
	M 2.....	I	I	7
		2	I	I	
		3	3	
		I	I	
	M 3.....	..	I	I	3
		I	I	
		I	I	
	M 4.....	..	I	I	3
		I	I	
		I	I	
	M 5.....	..	I	I	5
..		..	I	I		
..		2	I	I	..		
..		I	I		

TABLE 5 (Continued.)

Case.	Strain.	Tests Grouped under Lot of Inulin Medium Used.										Total Tests.			
		Tubes Inoculated.					Results.								
		1st Ox Ser. Lot.			2d Ox Ser. Lot.		Coagulation.								
		1	2	3	4	5	-	SL	+	++	+++				
Ns3 ₁₁₉₂	R. B.....	{	1	1	..	5		
		{	1	1			
		{	3	..	2	..	1	..			
	MR 1.....	{	..	1	1	4		
		{	3	2	..	1	..			
		{	3	2	1	..			
	MR 2.....	{	1	1	..	3		
		{	1	1	..			
		{	1	1	..			
	Total.....			47	13	..	18	12	4	47
	Tests Grouped According to Ox Serum used.	1st Ox Serum.....	{	3	1	2	
			{	..	5	5	
			{	1	..	2	5	2	
2d Ox Serum.....		{	18	7	..	2	8	4	18		
		{	11	5		
		{	3	..	4	..	9	2		
			29	4	..	18	7	..	29	

TABLE 5 (Continued.)

Case.	Strain.	Tests Grouped under Lot of Inulin Medium Used.											Total Tests.	
		Tubes Inoculated.						Results.						
		1st Ox. Ser.			2d Ox. Ser.			Coagulation.						
		Lot.			Lot.									
		1	2	3	4	5		—	S	L	+	+	++	
N 911222	Orig.....	I	I	6	
		..	I	I		
		I	I		
		I	2		
	M 1.....	I	I	2	
		I	..	I		
	M 2.....	..	I	I	3	
		I	I		
	M 3.....	I	..	I	5	
		3	I	I	..	I	..		
		3	3	..		
	R. B.....	I	I	..	3
		I	I	..		
		I	I	..	
	MR 1.....	..	I	I	..	4
		2	I	I	
	MR 2.....	I	I	..	7
		3	2	I	..	
	M 3.....	2	2	..	1
		I	I	
	M. D.	I	I	4
		2	2	
	MM 1.....	I	I	..	8
		4	3	I	..	
		3	3	
			I	I	..	

TABLE 5 (Continued.)

Case.	Strain.	Tests Grouped under Lot of Inulin Medium Used.											Total Tests.												
		Tubes Inoculated.						Results.																	
		1st Ox Ser.			2d Ox Ser.			Coagulation.																	
		Lot.			Lot.																				
1			2			3			4		5		—					SL		+		+		++	
N9I ₁₂₂₂	MM 2.....	{	..	1	1	{	6									
			2	1	1												
			1	1												
			2	1	1	..												
	Total.....	52	14	1	5	27	8	52												
Tests Grouped According to Ox Serum used	2d Ox Serum	{	1	1	{	..										
			..	6	4	..	1	1										
			19	2	1	1	10	5		..										
	1st Ox Serum.....	{	26	7	1	2	11	8	26											
			14	3	..	2	9											
			12	..	4	..	1	7											
			26	7	..	3	16	..	26											

A few cultures of streptococci recently obtained from various pyogenic sources were inoculated into animals, and the morphology and inulin growth in media studied as above. One of these strains resembles a pneumococcus, and one a *Pneumococcus mucosus* in several cultures, and all show capsules after passage through animals. Table 6 gives a summary of results and shows that all inulin cultures remain negative. These experiments are too few to be of any importance, but are given as controls, and because it seems possible that further study in this direction may establish the pneumococcus nature of some organisms now classed as streptococci.

TABLE 6.

Synopsis of Characteristics of Streptococcus Cultures Before and After Passage Through Animals.

Case.			Original Strain.	Rabbits,	Mice.					
					M.A.	M.B.	M.C.	M.D.	M.E.	M.F.
* Trachoma.....	Morphology	Typ.	+	+	+	±	±	±	±
		Atyp.	+
		Caps.	+	-	+	+	+	+	+
		Inulin	-	-	-	-	-	-	-
† Empyema.....	Morphology	Typ.	+	±	+	±	+	
		Atyp.	Lived
		Caps.	+	+	+	+	
		Inulin	-	-	-	-	-	
Puerperal Septicæmia	Morphology	Typ.	+	+	+	
		Atyp.	Not inoculated
		Caps.	+	?	
		Inulin	-	-	-	

Tests of the various lots of inulin powder and of the inulin medium before inoculation have so far led to no explanation of the varying coagulation results. No differences could be detected between the

* Many cultures suggest resemblance to *Pneumococcus mucosus*, most marked in cultures from Mouse A.

† Cultures from rabbit and from Mouse B suggest resemblance to pneumococcus.

several stocks of inulin powder, the rejected stock appearing to be identical with the rest except that coagulations could not be obtained with the medium made from it. Nine lots of the inulin medium made at different dates from good powder were tested for acidity before inoculation with organisms. Five c.c. titrated with N/50 NaOH and phenolphthalein were found to vary between neutral and 1.4 acid, the majority being about 0.2 acid.

Flasks containing 50 c.c. of inulin medium after titration for initial acidity were inoculated each with 5 c.c. of one of the cultures of the strains studied, and inoculated for 10 days. Some of the contents of these flasks was pipetted out every one or two days and tests made of reactions and growth in the inulin medium. Two flasks inoculated with N 9I₁₂₂₂ showed coagulation nearly complete in 24 hours, with acidity 2.2 and 2.8 respectively, organisms very characteristic, and no further tests possible because of coagulation. All flasks inoculated with other organisms gave a negative coagulation result, and showed practically no increase in acidity, while at the same time the count of plate colonies showed a good growth of organisms present. Table 7 gives the results of these tests with four of the previously mentioned strains and also with one strain of streptococci; the highest acid production being given in each case compared with the reaction before inoculation, and with the highest plate count.

TABLE 7.

Results of Titration and Plate Count with Non-Coagulation of Inulin Flask Cultures—5 c.c. Titrated with N/50 NaOH.

Strain.	Titration.		Plates.
	Before Inoculation.	Highest Acidity.	Highest Count to 1 c.c.
16 ₁₁₁₉	1.4	1.9	98,102,440
47 ₁₀₀₁	1.1	1.7	128,142,000
N23 ₁₁₂₂	0.4	0.9	45,896,050
N62 ₁₁₂₂	0.2	1.7	73,710,000
Streptococci.....	0.2	1.8	33,015,000

Further work in this direction was contemplated but has not yet been carried out, the same being true of plans to attempt to ascertain the nature of the acid produced by the growth of the pneumococcus in the inulin medium.

Conclusions.

Many strains of pneumococci, after longer or shorter periods of cultivation on artificial media, are found to undergo decided changes in morphology, virulence, and power to ferment inulin.

These changes may be temporary, disappearing when the organisms are placed under favorable conditions, but in some cases they seem to be permanent, the organisms having apparently undergone a complete change from their original characteristics.

The change in morphology is toward a more or less complete approach to a streptococcus type, some tendency in this direction appearing in certain cultures of every organism studied.

The presence of the pneumococcus and of the *Pneumococcus mucosus* types in organisms of the same strain shows the close relationship and interchangeable character of these two types.

The change in virulence corresponds with that usually found after long artificial cultivation. By transfers directly from animal to animal a rapid increase of virulence is developed.

The change in inulin fermenting power represents a marked irregularity of reaction constituting the chief feature in this series of tests which, including those made at time of isolation, consists of 452 inulin tests made with cultures from 63 strains of organisms.

A large number of negative tests have been given by very typical pneumococci and, on the other hand, many coagulations have been obtained from cultures of a definite streptococcus type.

Great variations have been found between different stocks of inulin powder and also between different lots of inulin medium made from the same powder and inoculated with organisms of the same strain.

Passage through animals seems, in many cases, to have a favorable effect upon the inulin fermenting power of the pneumococcus.

The use of strong emulsions of organisms sometimes produces coagulation, where ordinarily abundant cultures give only negative results.

The conclusion drawn from these experiments is that while coagulation of inulin is valuable corroborative evidence in favor of the pneumococcus nature of an organism, yet the irregular nature of the reaction may make it a fruitful source of differences and errors in diagnosis if too much reliance is placed upon this test. It is evident that no organism can be rejected as a pneumococcus because of one, or even of several, non-coagulating inulin cultures. Especially is this true of cultures which have been grown for some time on artificial culture media.

LABORATORY NOTES ON SPIROCHAETA OBERMEIERI FOUND IN NEW YORK.

ADELE OPPENHEIMER, M. A.

The spirochete studied by us was originally obtained by Dr. Norris from the blood of a patient in the service of Dr. Carlisle² in Bellevue Hospital. This organism was grown by Dr. Norris in white rats. Through the kindness of Dr. Flournoy, pathologist to Bellevue Hospital, we received some spirochetal rat's blood; thereafter the parasite was kept alive by means of passage from rat to rat according to the method of Dr. Norris,⁸ Director of Laboratories of Bellevue and Allied Hospitals.

In the proceedings of the New York Pathological Society, December, 1905 (p. 163) Dr. Norris reported that he had succeeded in growing the spirochete in the blood of rats, the first time in history that *Spirillum Obermeieri*^a had been grown in this animal. Some of this spirochetal blood he kindly gave either directly or indirectly to various workers and we herewith wish to report in what respects our observations and conclusions agree and disagree with those not only of Norris and his associates,⁸ but also with those of Novy and Knapp⁹ and of Breinl (Lancet, 1906, June 16, No. 4320, p. 1690).

Historical Sketch.

The papers of Schaudinn and Hoffmann on *Spirochaeta pallida*^b aroused a fresh interest in *Spirochaeta Obermeieri*. Investigations of the latter had been carried on ever since Obermeier, in 1873, reported the discovery of this organism in the blood of those suffering from relapsing fever. Through these long years much had been learned concerning this parasite, as can be seen by consulting any text book on bacteriology or by perusing the masterly articles of Wladimiroff¹¹ and Hödlmoser.⁴

^a It is not definitely known whether the New York spirochaete is *Spirillum Obermeieri* or not.

^b *Arbeiten kaiserl. Gesundheitsamte*, Berlin 1905, April 10, xxii. *Zweites Heft*. 527; *Deutsche Med. Wochenschr.*, 1905, May 4, xxxi, p. 711.

Until very recently it was classed with the bacteria, but now owing to the work of Schaudinn and others, one of the questions to be solved is whether the spirillum of Obermeier is or is not a protozoon.

The *Spirochaeta Obermeieri* (New York) was observed by us in the rat, in vitro, on the slide, and in the hanging drop. Under these heads we desire to call attention to such of our observations as we consider have not been sufficiently emphasized by others, such as have not been recorded by others, such as differ from those of others, such as have led us to disagree with the conclusions of others.

The smears were stained with Giemsa's or Wright's or Goldhorn's blood stain.

In the Rat.

1. It has been claimed by some observers that the length of time that the spirochetes are found in the blood constitutes one of the chief points of difference between the *Spirochaeta Duttoni* of African tick-fever and *Spirochaeta Obermeieri*, and indicates that each of the two organisms represents a distinct species.

As a result of subcutaneous injection according to our method of procedure, the parasites usually appeared in the blood of the tail within 96 hours after inoculation; disappeared *after* 24 hours, and did not reappear; *and this was true from the very beginning of our work in November and December, 1905.* Our work was stopped in March, 1906.

In our rat 26^c the facts on hand might be interpreted as fifteen days' presence of the organism in the peripheral blood; they *must* be interpreted either as more than three days' presence or as more than two relapses. Not Norris, Pappenheimer and Flournoy,⁸ nor Novy and Knapp⁹ nor we know that there are relapses in rats infected with *Spirochaete Obermeieri* (New York); if this is the case, we are not aware of the fact.

According to Breinl (Lancet, 1906, No. 4320, p. 1690)^d there are never more than two relapses in rats inoculated with the New York

^c See Table I.

^d See also Breinl and Kinghorn, p. 48 (7).

strain, and the maximum presence is three days for the New York strain and 17 days for the African strain.

See our rats 21, 26, 42, 49, in which the parasites were apparently found in the circulating blood for more than three days. Norris and his fellow workers,⁸ too, state that the New York spirochete is in the peripheral blood of rats 1 to 5 days. And in May, 1906, Novy and Knapp record that "as a result of the consecutive passage of the spirilla through this long series, increase in virulence, if this expression can be used, was noted. . . . Usually, however, the spirilla now disappear in about 60 hours after inoculation. Exceptionally a rat is met with in which the period of infection lasts three or four days."

However, in *January*, 1906, Novy made the following statement in the "Journal of the American Medical Association" (vol. xlv., No. 2): "The persistence of the spirochetes of tick-fever in the blood of rats for 3 to 8 days, as shown by Dutton and Todd, would indicate that their organism, though closely related, is nevertheless different from that studied by us. It goes to show that the tick-fever of Africa and the relapsing fever of Europe are due to different species of spirochetes."

Either our rats are not like Novy's, or our method of passage is unlike his in points that are essential. As a matter of fact he injected intraperitoneally, we subcutaneously^e; and evidently he injected more organisms than we. That a difference of method can influence the day of appearance of the parasites in the blood is shown by the fact that whenever our blood was not used at once, but kept a day before using it for inoculation, the appearance, too, of the spirilla in the circulating blood of the inoculated rat, was delayed. In the second place, it should be noted that under these conditions the parasite was never found in the peripheral blood more than two days in succession^f. We have not attempted to learn whether there is a loss in the number of living spirochetes or in their vigor when the diluted blood stands a day.

^e Except in cases of rats 3, 4, 5 and 6, which were injected intraperitoneally.

^f Breinl and Kinghorn (7), state of *Spirochaeta Duttoni*: "the incubation period varied directly with the amount of infected blood injected and with the mode of inoculation. The parasite multiplied more or less rapidly depending on the dosage."

Such an investigation is, of course, difficult to carry out with organisms that one has not succeeded in cultivating on artificial media.

These studies show that more work needs to be done before the relationship between the New York and African strains can be determined (see also No. 8, p. 144 and No. 3, p. 142^g).

2. The symptoms of the rats were not studied, but it was noted that not a few of the rats were restless when they had spirochetes in the circulating blood, just as they are when affected with other diseases. This restlessness helped us to pick out the rat with spirochetal blood on any one day^h.

3. Rat 26 apparently died of spirillosis. Novy and Knapp⁹ particularly state that they had no such deaths.ⁱ Of all our rats this was the only one which did not keep itself clean.

4. Sometimes the parasites were present in great numbers and at others could only be found by searching several microscopic fields; and these variations were, too, apparently without any law or order. Such, as well, is the case in European relapsing fever in man.

5. Rats 8 and 25 injected with blood the day after the disappearance of the organisms from that blood did not become infected. (See Conclusion 5, p. 146.)

6. Rats 12 and 15 injected with blood two days before the appearance of the spirochetes in that blood did not become infected. (See Conclusion 5, p. 146.)

7. In rat 47 the inoculation of living organisms did not result in an infection. Was 47 more resistant than the average white rat?

According to Novy and Knapp,⁹ another plausible explanation for these "resistant" exceptions is the injection of only a few living spirochetes and a large dose of immune bodies. This explanation might hold for our rats 51 and 52; but it does not hold for 47, since though inoculated with the same material on the same day 48 became infected.

^g Breinl (*Lancet*, 1906), states that the spirochaete of the African tick-fever is of a species differing from that of the New York spirochaete in that each confers a relatively active immunity against itself but not against the other.

^h The rats were in charge of Mr. J. Behan, Laboratory Assistant.

ⁱ So also Breinl and Kinghorn, p. 49 (7).

8. The presence or absence of the spirochetes in the spleen is particularly important in the light of Metchnikoff's findings in the case of the relapsing fever of Europe. He found phagocytic destruction of the organism in the spleen during the apyrexial period.

At autopsy, judging from smears, the spirilla were present in the peripheral blood and in the heart only. They were never present in the spleen, kidney, suprarenal body, lung, or liver (see Table IV.). Our results here agree with those of Norris, Pappenheimer and Flournoy⁸ and disagree with those of Novy and Knapp⁹ who found spirochetes in all the organs of the rat.

It should be noted that rat 31 was dissected before and 34 after the appearance of the parasites in the circulating blood.

Though rat 62 showed organisms in the blood of the tail on February 6th, at autopsy on that day no organisms were found in the heart's blood. Perhaps the height of the infection had not yet been reached. Perhaps rats 51 and 52 did not become infected because they were injected with the blood of rat 49 before the climax in 49 was reached.

In Vitro.

1. One of the chief aims of workers with *Spirochaeta Obermeieri* is to grow these on artificial culture media.

In vitro the organisms remained alive in rat's blood, but as far as our work is concerned the various media have proven themselves to be simply the support on which or in which the rat's blood rested. The spirillum merely held its own in vitro, it increased slightly if at all, and we cannot say that we had a culture.

In one instance, we moved^j successfully, the blood containing spirochetes from the heart to test-tube 1; then, on the second day after, the spirochetes from test-tube 1 to test-tube 2 (containing normal rat's heart's blood shaken); again, on the second day after that, the spirochetes from test-tube 2 to test-tube 3; but not to test-tube 4. In another instance, not even the removal to test-tube 3 was successful. In a third case, we moved the blood containing spirilla from the heart

^j We use the word "moved" in preference to "transferred" in order to avoid the connotation of growth.

to a test-tube containing a slope of sheep-serum glucose agar, then, on the eighth day after that, the organisms to test-tube 2, but as we had a contamination we had to stop short. Finally, the removal from potato, test-tube 1, to potato, test-tube 2, but not to potato, test-tube 3, was successful.

Working also with the New York strain, Norris and his collaborators⁸ believed they had cultures of the first and second generation, outside the body of the animal. Novy and Knapp⁹ report a lack of success on their part outside of the body of the animal but growth of the parasites within capsules in the peritoneal cavity of white rats.¹⁰

2. Incubator temperature seems to be unfavorable to long life of the organism when outside the body of the host ^k.

3. The interpretation of the well-known tangles and intertwinings of the organisms varies with different authors.

In smears of material that had been in vitro, the spirillum often appeared more slender.^l These slender forms suggested the stage of *Spirochaeta pallida* interpreted as pre-microgametic by Krzysztalowicz and Siedlecki.⁶ Here the organism was found in tangled groups as well as singly. Here also one often saw two (or perhaps more) spirochetes interwoven. The tangled masses might be explained as the result of agglutination, especially as the organisms composing them and surrounding them were particularly long, except for the fact that (in the case of 38 sheep-serum glucose agar) no large groups were seen until some days had elapsed.

According to Novy and Knapp⁹ the presence of tangles as observed by Koch (in insect and egg) indicates an agglutinated condition and not multiplication. On the other hand, Levaditi is apparently of a different opinion, as can be seen from the following quotation: "Dass es sich hier nicht (chicken spirochaeta) um eine Agglutination des Spirochäts handelt hat Levaditi durch mikroskopische Beobachtung des Blutes bei 38° festgestellt, wobei er die Haufen in 4-35 Minuten sich

^k According to L. A. and R. S. Williams (7) room temperature is also the best for the continued existence of *Spirochaete Duttoni* in vitro.

^l In "cultures" of *Spirochaeta Duttoni* made by L. A. and R. S. Williams (7), "the spirochaetes occasionally appeared to be thinner than normal."

wieder in einzelne freibewegliche Spirochäten auflösen sah." (Quoted from Wladimiroff.)

Koch suggests in the "Berliner klinische Wochenschrift," February, 1906, that chemiotaxis may account for the clumps and recalls the fact that similar plaited masses are to be seen in the flagella of bacteria.

It is not unlikely that the tangles and intertwinings are due to the concentration or coagulation of the blood on standing, or the intertwining to fusion or conjugation. One of the intertwined spirochetes is always coarser than the other (or others), though none is as plump as the "bands" mentioned in paragraph 2 of the next section.

It is unlikely that a longitudinal split should account for these intertwinings; for longitudinal fission would probably result in two organisms, lying side by side and that for more than a small part of their length. The spirilla, in all probability, increase not by longitudinal but by transverse division as is indicated by transverse breaks and faintly stained attenuated areas.

On the Slide.

1. Instead of the typical spiral, one not infrequently sees whiplike organisms. Are these whips and individuals with sweeping waves due to the method of fixation?

2. One also sees certain straightened and flattened bands recalling the involution forms of the bacteria and suggesting, because of their shape, the so-called macrogametes of *Spirochaeta pallida* as described by Krzysztalowicz and Siedlecki⁸. In some of these thick forms there was an unstained cleft which might be interpreted as the beginning of longitudinal division. The "band-like" form as a whole may stain quite intensely, but usually it is pale. Are these "bands" crushed organisms?

3. The spirillum is straight or curved, or more seldom U-shaped or about ring-shaped, or still less frequently tangled or knotted. We do not agree therefore with Novy and Knapp,⁹ who declare that the tendency to figure 8 forms or even perfect circles does not (as in *Sp. Duttoni* and relapsing fever of Bombay) seem to exist in the New York *Spirochaeta Obermeieri*. In the hanging drop of the unidenti-

fied slender spirillum (mentioned below—from the fæces of a dog) the organism was seen to take on tangled shapes.

4. The New York spirochaeta is almost invariably gradually drawn to a point at each of the ends. These two faint terminations resemble the pale attenuated median area mentioned below and do not suggest organoid cilia or flagella, but rather prolongations of the periplast of the organism.

On some few spirilla indications of a flagellum like that described by Novy and Knapp^m were noted. No flagellum-stain was triedⁿ.

5. Generally on the slide the spirochete touches a red blood cell or two, more rarely a white blood cell or a blood plate, broadside or with one or the other or both ends. Sometimes it terminates bluntly against a red blood cell; is it then dipping into the cell? But perhaps all these connections come about in making the film, for we have never seen any such in the hanging drop.

Norris and his fellow-workers⁸ noted, when immune and spirochetal blood combined were watched in vitro, a marked tendency of the organisms to adhere to the red blood corpuscles.

6. The parasite is often more or less beaded, and then looks as if irregularly stained, as bacteria frequently are. The beading may of course, also, be either an artefact or the result of degenerationⁿ.

7. A grain-like, deeply stained body was seen in one spirillum, which since it occurred in a smear of normal spirochetes may not have been an artefact, but perhaps the result of degenerative processes.

8. The significance of the transverse breaks is a mooted question. The organism is usually $1\frac{1}{2}$ times the length of a red blood cell, or $2\frac{1}{2}$ times the length of a red blood corpuscle, with a transverse break at about the middle. That we have not here two organisms lying end to end is indicated by the flat surfaces which bound the transverse break. Indeed these long "broken" forms of the parasite predominate

^m See E. Zettnow's demonstration in the case of *Sp. Duttoni*, of flagella like those of bacteria, recorded in *Deut. med. Woch.*, xxxvi, 10, March 8, 1906.

ⁿ For a note on the frequent occurrence of such granulations in spirochaete-like organisms see Dutton, Todd, and Tobey, 7.

in the early stages of the infection and therefore probably before agglutination could be said to bring organisms together. Two organisms lying end to end are not infrequently seen.

In addition to the transverse breaks, one sees similarly located, lightly-stained, attenuated areas. Such a pale, thin zone resembles the faint tips, the two ends of the spirochetes which fade away to a point.

These forms which are pale and thin or broken at the middle of their length might be interpreted as the final stages in a longitudinal division, but definite corresponding early phases have not been observed, namely, the Y-shaped and U-shaped individuals with the attenuated area at the bend of the U.

Novy and Knapp⁹ state that several division zones like those of the Bombay organism and like those present in *Sp. Duttoni* are not to be found in the New York *Sp. Obermeieri*. On the other hand, we have seen as many as four faint or unstained spots (breaks or attenuated portions) in the course of a single individual, sometimes cutting off from the rest of the spirillum only two, or even only one wave (hinting at vibrio-form or microgametes). These "single" (?) individuals are considered by some a string of individuals.

The modes of reproduction more clearly suggested by these appearances are transverse fission and fragmentation. In support of the view that the mode of reproduction is not longitudinal, but transverse, is the fact that even in the early stages of the infection, not short, thick forms, but long ones predominate.

In the Hanging Drop.

1. Ultimately it is in the hanging drop where the process of multiplication must be studied and there we have not yet seen any division.

Novy and Knapp⁹ have often actually seen transverse division in the hanging drop, and Norris, Pappenheimer and Flournoy⁸ believe they have seen it once; but all these workers point out the fact that these observations are capable of other interpretations.

2. The differences in the description of the motility of the *Spirocheta Obermeieri* are probably due to differences of the conditions under which the parasites are watched.

In the mucus of the fæces of a normal dog were found two kinds of spirochetes, namely, a slender and a thick one^o. The slender one moved like a snake, its curves were seen to change; in the smears there were far less and broader curves than in the drop. The thick one moved by means of beating its way through the water and apparently by means of a corkscrew motion, its curves were fixed; in the smears there were as many curves as in the drop. The New York Sp. *Obermeieri* is more like this latter organism than like the former; though it is flexible, its coils are permanent. We have never seen it when it was moving as quickly as either of them. We have seen it swing one end^p and move from place to place seemingly by means of a corkscrew motion. All these three organisms move with either end forward. The *Spirochaeta Obermeieri* New York travels in either direction, with the characteristic pauses, gradually or suddenly out of the microscopic field. The corkscrew motions and undulations are more rapid than the progression; indeed the organism often stands still, fastened perhaps to the cover glass, and meanwhile bends slowly and usually undulates rapidly.

There pass over the *Spirochaete Obermeieri* undulations which persist in preparations longer than any other of its motions and which may be due to an undulating membrane. Perhaps, on the other hand, this pennant-like waving is a mere appearance and not a reality, for the rotations of a spiral about a long axis would simulate a wave motion in one plane as Norris and his co-workers⁸ have pointed out. Perhaps the spiral of *Spirochaete Obermeieri* is rigid except for the lateral swayings. It is impossible to tell which of these phenomena are passive, which locomotor.

^o These were found by Mr. Thomas Deaken, laboratory assistant.

^p "The swaying lateral motion from side to side is seen only in the long forms, which consist of two or more cells. This is also a secondary condition to the real motion, and although it imparts to the long spirochete the so-called flexible character, the latter feature is hardly of sufficient importance to justify its employment as the basis of a generic difference among the spiral organisms. The long form of the *Spirillum rubrum* or of the cholera vibrio, as is well known, will show similar lateral swayings" (Novy and Knapp⁹).

Conclusions.

1. The New York Spirochaeta Obermeieri cannot yet, as has been attempted, be separated from the African spirochete, upon the following grounds: (1) the length of its stay in the peripheral blood of the rat, (2) the number of relapses in the rat, (3) the lack of figure 8 and circular forms, (4) the absence of several transverse breaks; for the length of stay in the peripheral blood probably varies with the method of passage relapses are an uncertain quantity since it is perhaps not positively established that they occur at all figure 8 forms and circles and finally several division zones exist in the New York spirillum as well as in Sp. Duttoni and in the spirillum of Bombay.

2. As far as our work is concerned the parasite merely holds its own in vitro; we cannot say that we had a culture.

3. It is not unlikely that the tangles and intertwinings, seen during attempts at cultivation on artificial media, are due to the concentration or coagulation of the blood on standing, or the intertwinings to fusion or conjugation.

4. The Spirochaete Obermeieri probably increases by transverse fission and fragmentation.

5. We have seen no evidence of sporulation (no spore stain was used) or of a cycle of development, unless the particularly slender forms, short forms, "bands," and intertwinings be considered such evidence. The only host studied was the rat.

6. The variations in the description of the motility are in all likelihood due to differences in the conditions under which the parasites are watched. As observed by us, its motility is almost precisely like that described by Hoffmann,⁵ for Spirochaeta pallida.

7. Perhaps the undulations that pass over the organism are merely an appearance and the spiral is in truth rigid except for the lateral swayings.

8. The indication of an undulating membrane in the hanging drop is the only sign of a definite structure which we have seen, except a deeply stained grain (mentioned above). The absence of a complicated structure, the apparent multiplication by transverse division and fragmentation, the rapidity of multiplication, the length of viability

outside of the body, and the persistence of the spiral form in death, point to a bacterium; whereas the flexibility of the parasite, the indication of an undulating membrane, the inability to cultivate the organism on artificial media, and the death at incubator temperature suggest that the New York *Spirochaeta Obermeieri* may be a protozoan ^q.

According to Schaudinn, *Spirochaeta pallida* is not a spirochete and not a spirillum but a treponema. It is not a spirochete because of the permanency of its coils, because of its terminal cilium, and because it has not more or less blunted ends. It is not a spirillum because its spirals are flexible, because it has a single cilium instead of a terminal tuft, because it apparently divides longitudinally. The New York *Spirillum Obermeieri* is certainly flexible and has permanent coils; if against our better judgment we grant in addition that each of its two ends represents a cilium and that the parasite divides longitudinally, then the New York *Spirochaeta Obermeieri* must be classed with *Spirochaeta pallida* as a *Treponema*.^r

The three tables which follow show the number of examinations made in the case of each rat, and the number of days on which spirochetes were found in the blood of the tail of these rats.

For inoculation, blood was drawn from the infected rat's tail into a syringe containing 10 per cent. sodium citrate solution.

^q The tranverse mode of division also occurs among the protozoa; indeed no one of the facts mentioned is in itself a sufficient basis for classification.

^r Hoffmann believes that there are now no grounds for separating *pallida* entirely from the other spirochetes and giving it the specific name of *Treponema*, since other spirochetes, for instance *balantidis*, too, have a terminal filament and permanent coils.

TABLE I.

Of Rats Injected with Fresh Blood.

Rats.	The Inoculations into These Rats Were Made from the Following Rats.	Dates of Inoculation.	Dates on Which the Peripheral Blood Was Examined.	Dates on Which the Spirochetes Were Found.
5.....	Bellevue	November 28	29, 1, 2, 3.....	2.
4.....	5	December 2 {	From December 3 to January 23 {	December 7 and 8 and not again thereafter.
6.....	4	December 7	8, 9, 10, 11.....	10.
7.....	4	December 7	8, 9, 10, 11, 13.....	10 and 11.
8.....	4	December 9	11, 12, 13, 14, 15.....	0 ^a
12.....	11	December 15	16, 18, 19, 20, 21.....	0 ^a
13.....	10	December 15	18.....	0 ^a
18.....	11	December 19	21, 22, 23.....	22, 23.
19.....	11	December 19	21, 22, 23, 24, 25.....	23 and 24.
20.....	18	December 22	24, 25, 26, 27, 28.....	26 and 27.
21.....	18	December 22	24, 25, 26, 27, 28, 29, 30, 31.....	27, 28, 29, 30, 31.
22.....	19	December 23	25, 26, 27, 28, 29.....	26, 27, 28.
23.....	19	December 24	26, 27, 28, 29, 30.....	28 and 29.
24.....	20	December 26	28, 29, 30, 31.....	29 and 30.
25.....	20	December 28	30, 31, 1, 2, 3, 5.....	0 ^a
26.....	25	December 29 {	31; 1, 2, 3, 4, 5, 6, 8, 9†, 10, 11, 12, {	3, 4, 6, 8, 10, 11, 12, 15, 16, 17, 18.
27.....	25	December 30	15, 16, 17, 18†. Dead on 19th.. }	3 and 4.
28.....	26	January 3	1, 2, 3, 4, 5.....	8.
29.....	26	January 4	5, 6, 8, 9..	8, 9, 10.
30.....	26	January 6	6, 8, 9, 10, 11.....	11.
31.....	28	January 8	8, 9, 10, 11, 12.....	0. Killed on January 9.
32.....	28	January 8	9.....	12. Killed on January 12
33.....	28	January 8	10, 11, 12.....	0. †
34.....	29	January 10	10, 11, 12, 13, 15, 16.....	15, 16, 17. Killed on January 18.
35.....	32	January 12	12, 13, 15, 16, 17, 18.....	17, 18.
37.....	34	January 15	15, 17, 18, 19.....	19 and 20.
38.....	35	January 17	18, 19, 20, 22, 23.....	22. Killed on January 22.
39.....	37	January 19	19, 20, 22	0. †
40.....	36	January 20	22, 23, 24	23 and 24.
41.....	38	January 22	22, 23, 24	25. Killed on January 25.
42.....	40	January 23	24, 25.....	26, 27, 29.
43.....	41	January 25	25, 26, 27, 29, 30.....	29.
			26, 27, 29, 30.....	

Rats.	The Inoculations into These Rats Were Made from the Following Rats.	Dates of Inoculation.	Dates on Which the Peripheral Blood Was Examined.	Dates on Which the Spirochetes Were Found.
44.....	42	January 27	29, 30, 31, 1.....	29, 30, 31.
45.....	42	January 29	31, 1, 2, 3, 5, 6.....	3, 5.
46.....	44	January 31	11, 2, 3, 5, 6.....	3, 5. Dead on February 15.
47.....	46	February 3	5, 6, 7, 8, 9, 10.....	0. †
48.....	46	February 3	6, 7, 8.....	7, 8. Killed on February 8.
49.....	48	February 7	9, 10, 13, 15, 16.....	10, 13.
50.....	48	February 8	10, 13, 15, 16.....	13.
51.....	49	February 10	13, 15, 16.....	0. †
53.....	50	February 13	15, 16, 17.....	16, 17. Killed on February 17.
54.....	53	February 16	17, 19, 20.....	20.
55.....	53	February 17	19, 20.....	19, 20.
56.....	53	February 17	19, 20, 25.....	0. †
57.....	55	February 19	20, 23.....	23. Killed on February 23

* Rats 8 and 25 were inoculated with blood after the disappearance of spirochetes from that blood; and rats 12 and 13 were inoculated with blood before the appearance of spirochetes in that blood.

† In the case of rat 26, the smear of the ninth was poor, after that day the smears were made not from the tail but from the ear. The smear of the eighteenth was poor.

‡ Rat 33 was not examined on the 14th; rat 39, not on the 21st; rat 51, not on the 12th and 14th; rat 56 was not examined on the 21st, 22d, 23d, 24th.

TABLE II.

Of Rats Injected with Blood One Day Old.

Rats.	The Inoculations into These Rats Were Made from the Following Rats.	Dates of Inoculation.	Dates on Which the Peripheral Blood Was Examined.	Dates on Which the Spirochetes Were Found.
9.....	6	December 11	12, 13, 14, 15.....	o*
10.....	7	December 11	12, 13, 14, 15, 16.....	o*
11.....	7	December 12	13, 14, 15, 16, 18, 19, 20.....	18, 19.
14.....	10	December 16	18.....	o*
15.....	11	December 16	18, 19, 20, 21.....	o*
36.....	32	January 13	15, 16, 17, 18, 19, 20, 22.....	19, 20.
52.....	49	February 11	13, 15, 16, 17.....	o*
58.....	54	February 21	{ Dead February 23.
59.....	54	February 21	23, 25, 27, 28, 1.....	
60.....	57	February 24	{ Dead February 24.
61.....	57	February 24	27, 28, 1, 2, 3, 4.....	
62.....	59	March 1	2, 3, 4, 5, 6.....	6. Killed March 6.

* Rat 9 was not examined after the 15th; and rat 10, not after the 16th. Rats 14 and 15 were inoculated with blood before the appearance of spirochetes in that blood. Rat 52 was not examined on the 14th. Rats 58 and 60 were young, but so too was rat 57. Rat 61 was very large.

TABLE III.

Of Rats Injected with Blood More than One Day Old.

Rats.	The Inoculations into These Rats Were Made from the Following Rats.	Dates of Inoculation.	Dates on Which the Peripheral Blood Was Examined.	Dates on Which the Spirochetes Were Found.
16.....	3+4+6+7	December 18	20, 21, 22, 23.....	o.
17.....	3+4+6+7	December 18	20, 21, 22, 23.....	o.
63.....	59	March 3	5, 6, 7, 8, 9, 10, 12, 13, 14, 15.....	o.
64.....	62	March 10	12, 13, 14, 15, 16, 17.....	o.

TABLE IV.

Of Rats on which Autopsies Were Made.

Rats.	Autopsy Made on	Peripheral Blood: Spirochetes Present (+) or Absent (-).	Heart: Spirochetes Present (+) or Absent (-).	
26*	January 19	..	+	No spirochetes in smears of the spleen, kidney, suprarenal body, lung or liver. No spirochetes in smears of the spleen, kidney, suprarenal body, lung or liver. No spirochetes in smears of the spleen, kidney, suprarenal body, lung or liver. No spirochetes in smears of the spleen, kidney, suprarenal body, lung or liver. No smears made of any of the organs except the heart. No smears made of any of the organs except the heart. No smears made except of the heart. Rat 46 died after not having been examined since the 6th. No spirochetes in smears of the spleen. No smears made of any of the organs except of the heart and spleen. No smears made of any of the organs except the heart. No spirochetes in smears of the spleen. No smears made of the organs except of the heart and spleen. No spirochetes in smears of the spleen. No smears made of any of the organs except of the heart and spleen.
31	January 9	—	—	
32	January 12	+	+	
34	January 18	—	—	
38	January 22	+	+	
41	January 25	+	+	
46	February 15	..	—	
53	February 17	+	+	
57	February 23	+	+	
58*	February 23	not examined	—	
62	March 6	+	—!	

* Rat 58 was a young rat inoculated on February 22 and found dead on February 23. In the case of rat 26 the smear of the heart's blood was poor. The blood was coagulated.

In concluding, I desire to express my thanks to Dr. William H. Park, Director of the Research Laboratory of the Department of Health, for the opportunity to do this work, and to Dr. Anna W. Williams, Assistant Director, for her helpful suggestions.

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COLLECTED STUDIES

FROM THE

RESEARCH LABORATORY

DEPARTMENT OF HEALTH

CITY OF NEW YORK

Dr. WILLIAM H. PARK, Director

VOLUME III.

1907



DEPARTMENT OF HEALTH

OF THE

CITY OF NEW YORK

DR. THOMAS DARLINGTON

Commissioner of Health

DR. HERMANN M. BIGGS

General Medical Officer

EUGENE W. SCHEFFER

Secretary

Research Soc. City 7-14-09

In the following pages have been collected all the papers published from the Research Laboratory in the past year, as well as a number of reports and protocols which were unsuited for publication in the regular technical journals. The recipients of this volume will confer a favor on their colleagues of the Research Laboratory by sending their own publications in exchange. Such pamphlets should be addressed to the Librarian, Research Laboratory, Foot of East Sixteenth Street, New York.

New York, September, 1908.

DR. CHARLES BOLDUAN,
Editor.

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THE OPSONIC INDEX AND THE USE OF BACTERIAL VACCINES IN THE TREATMENT OF DISEASE.

WILLIAM H. PARK and HERMANN M. BIGGS.

Assisted by Drs. Mary E. Goodwin, Charles Bolduan, Marie Grund, Arthur Kendall, and Miss Noble.

Introduction.

Historical—As far back as 1858 Haeckel had observed that particles of indigo injected into the veins of certain molluscs could shortly afterwards be found in the blood cells of the animal. However, the significance of this and other similar observations was not appreciated, until Metchnikoff, in 1883, called attention to their bearing on infection and immunity. The outcome of his investigations was the establishment of the well-known doctrine of *phagocytosis*, the principle of which is that the leucocytes attack and destroy the micro-organisms which have invaded the tissues. Metchnikoff's theory found but little acceptance, the more so since the studies of Nuttall, Pfeiffer, Behring, Ehrlich and others demonstrated a great variety of protective substances in the serum of immune animals. As time went on Metchnikoff realized that the serum played an important part in the destruction of bacteria, but he interpreted this action as being a stimulation of the leucocytes. Denys and Leclef showed that the increased phagocytosis in an immunized animal was due to an alteration in the serum.

In 1903 A. E. Wright, of England, discovered a certain substance in blood serum which united with the bacteria and rendered them more easily taken up by the phagocytic cells. He called this substance *opsonin* and showed that it was present in normal serum and in the serum of infected individuals, though in the latter, as will be discussed directly, it varied considerably from the average normal content. By means of absorption tests, he showed that the opsonin has a specific affinity for the bacteria and none for the leucocytes. Neufeld and Rimpau discovered the same point independently. The opsonins for the staphylococcus prepare only staphylococci, those for tubercle only tubercle bacilli, etc. If fresh, washed leucocytes are mixed with a bacterial emulsion, phagocytosis is very slow, so slow, in fact, that at the end of fifteen to

twenty minutes at 37 degrees C. the cells may still be empty. Under the influence of a serum containing opsonin the same leucocytes would have loaded themselves with bacteria, as can be seen by examining such a parallel control. It may be added that the leucocytes are believed by Metchnikoff to be the source of the opsonins. This author shows that the leucocytes act as phagocytes even without free opsonin, only it takes them longer to do it. The work of Hektoen seems to have clearly established that opsonins are distinct antibodies and not identical with bacteriolytic immune bodies. In the further study of these opsonins Wright developed the idea that they were highly important in combating a number of bacterial infections, and soon published observations on the opsonins in tubercle and staphylococcus infections. His results proved to him that inoculations of the corresponding bacteria produced marked changes in the opsonic content of the infected individual, and that it was possible to estimate accurately the immunizing effect of such inoculations. At the present time he has correlated all these observations and has attempted to build up a system of treating bacterial infections by means of active immunization controlled by opsonic measurements.

Technique—Wright's technique of measuring the opsonic power is a slight modification of the Leishman* method and is as follows: An emulsion of fresh human leucocytes is made by dropping twenty drops of blood from a finger prick into 20 c.c. normal salt solution containing one per cent. sodium citrate. The mixture is centrifuged, the supernatant clear fluid removed and the upper layers of the sedimented blood cells transferred by means of a fine pipette to 10 c.c. normal salt solution. After centrifuging this second mixture the supernatant fluid is pipetted off and the remaining suspension used for the opsonic tests. Such a "leucocyte emulsion," of course, still contains an enormous number of red blood cells; the proportion of leucocytes, however, is greater than in the original blood. One volume of this emulsion is mixed with one volume of the bacterial suspension to be tested and with one volume of the serum. This is best accomplished by means of a pipette made by drawing a capillary end to a glass tube. With a wax pencil mark about three-quarters of an inch from the end, it is easy to suck up one such vol-

* Leishman, British Medical Journal, January, 1902.

ume of each of the fluids, allowing a tiny air bubble to intervene between the different fluids. All these are now expelled on a slide and thoroughly mixed by drawing back and forth into the pipette. Then the mixture is sucked into the pipette, the end sealed in a flame, and the whole put into the incubator at 37 degrees C. The identical test is made using a normal serum in place of the serum to be tested. Both tubes are allowed to incubate fifteen minutes and then examined by means of smear preparations on slides spread and stained in the usual way. The degree of phagocytosis is then determined in each by counting a consecutive series of fifty leucocytes and finding the average number of bacteria ingested per leucocyte. This number for the serum to be tested is divided by the number obtained with the normal serum and the result regarded as the *opsonic index* of the serum in question. The presence of a high index Wright regards as indicative of increased resistance. He further states that fluctuations of the opsonic index in normal healthy individuals is not more than from 0.8 to 1.2, and that an index below 0.8 is therefore diagnostic of the presence of an infection with the organism tested. It should be stated that for the normal control Wright usually employs a mixture of several normal sera, thus seeking to secure a fair normal average.

Bacterial Inoculations—Wright's method of treating bacterial infections is based on the following premises: In localized infections the infected body absorbs but small amounts of bacterial substances or antigens. In consequence of this the amount of active immunity developed is but slight. Localized infections therefore tend to run a chronic course. The logical method of effecting a cure in these cases is to actively immunize the body with the invading organism. In a number of infections, notably those of staphylococcus, streptococcus and tubercle, the degree of immunity is measured accurately, according to Wright, by the opsonic power of the blood serum, *i. e.*, by the opsonic index. He believes it is almost impossible, by mere clinical observation, to determine whether or not the bacterial inoculations are producing increased immunity; that this is comparatively easy by means of opsonic measurements.

The bacterial inoculations consist of suspensions of agar cultures in normal salt solution. These suspensions are heated to 55 degrees or 60

degrees C. for twenty or more minutes to kill the organisms, and then receive small additions of carbolic acid or lysol as preservatives. Cultural tests are made to insure sterility, and the "vaccine" counted so as to determine the dose. This is readily accomplished by means of a small capillary pipette such as is used for the opsonic test. A wax pencil mark is made on the capillary tube and then one such volume of the vaccine to be tested, and one volume of blood taken from a fresh finger prick (an air bubble intervening) are sucked into the pipette. The two fluids are thoroughly mixed by sucking back and forth on a slide and the mixture then spread on a slide in the ordinary way for blood smears. After staining it is comparatively easy to ascertain, by counting through a ruled ocular, the proportion of bacteria to red blood cells. The latter being regarded as 5,000 million per c.c., it needs but a simple calculation to give the number of bacteria per c.c. So far as dosage is concerned one employs from 300 to 500 million in the case of staphylococcus, from 50 to 100 million in the case of streptococcus, etc.

Original Investigations.

Wright, it will have been seen, claims two fundamental points—first, that it is possible to determine the real opsonic power of the blood with sufficient accuracy to make it available for treatment, and second, that the opsonins are either the most important of the protective substances of the blood or that they undergo a sufficient proportional development with the latter to be a safe guide as to their amount.

The Accuracy with Which the Opsonic Power of the Blood can be Determined by Wright's Methods—The opsonic power of the blood is tested by mixing equal quantities of an emulsion of red cells and leucocytes, and an emulsion of bacteria and the serum. This mixture is held at 37 degrees C. for fifteen minutes and is then spread on the surface of a roughened slide. An examination of any slide will show that the different leucocytes vary in their size and in their content of bacteria. This is due partly to variation in phagocytic activity, and partly to the interference of the red blood cells, which are present in great numbers in the emulsion and separate the bacteria in different degrees from the white cells. These and other reasons to be mentioned bring it about

that the different leucocytes vary greatly in the number of bacteria they take up and in their distribution on the slide. To overcome this, large numbers of leucocytes are counted. Some enumerate the bacteria in fifty cells, others in one hundred or one hundred and fifty. Beyond one hundred, or at most one hundred and fifty, the increase of accuracy hardly compensates for the extra labor. The following table shows the difference between counting larger or smaller numbers of cells in five opsonic tests as determined by counting different numbers of cells.

Influence of Number of Cells Counted Upon Accuracy.

Cells Counted.	Average Number of Bacteria in each Leucocyte.				
50.....	1.18	1.88	1.34	1.42	1.90
100.....	1.22	1.78	1.24	1.42	1.59
150.....	1.18	1.62	1.22	1.44	1.50
200.....	1.18	1.51	1.22	1.46	1.37
600.....	1.28	1.62	1.23	1.36	1.36
1,200.....	1.34	1.44	1.25	1.30	1.42

It is necessary to have the counts that are compared all counted by the same person, as each individual has a somewhat different method and will average higher or lower for all counts than any other person.

It is noticed that the variation between the average cell count obtained from fifty cells and larger numbers is much greater than between that obtained at one hundred and larger numbers.

When two specimens of blood are tested, not only the inaccuracy of counting due to the different arrangement of the unequally filled cells on the slides to be counted is met, but the fact that in making the test the conditions are not similar, for in different mixtures slightly different proportions of leucocytes, bacteria and red cells will always be mixed together. If smears from a series of tubes of the same blood are compared with a series of smears from one of the tubes, the former will always show the greater variation.

This variation is much greater than most examiners believe. Dr. North collected a series of tests carried out in nearly all the important laboratories in the Eastern United States working upon opsonins. The results recorded seem to us to prove absolutely that while an average

counting error of only about 10 per cent. is present, there may be an exceptional error of at least 100 per cent., and one of at least 20 per cent. may be expected once in about every ten determinations.

The following is a fair average of the correctness of routine tests by experienced workers:

Absolute Count of Bacteria in One Hundred Leucocytes.

Blood Specimen A.		Blood Specimen B.		Blood Specimen C.	
Tube 1.....	156	Tube 1.....	142	Tube 1.....	89
“ 2.....	168	“ 2.....	182	“ 2.....	102
“ 3.....	172	“ 3.....	188	“ 3.....	121
“ 4.....	198				

This error, which occurs because of the technic, applies not only to the examination of the specimen of blood, but also to the measure we employ to estimate the amount of opsonins. As these are not stable, we cannot have a standardized solution, as we do with antitoxins. We must, therefore, determine our measure afresh in each test, taking for this purpose a supposedly normal blood. Wright, from a great many tests, has determined that the opsonic power of the blood in non-infected persons for tubercle bacilli does not vary, as a rule, more than 10 per cent. above or below the average power of healthy blood. For staphylococci there is more variation. It is found also that many things besides infection decrease the amount of opsonins in the blood. Hemorrhage, fatigue, starvation, and other influences which lower the resistance of the body have this effect.

Wright gets this measure as uniform as possible by determining the average opsonic strength of five supposedly healthy persons at the time of each test. If any one of these five is considerably below or above the others it is omitted for that day.

The measure so obtained will probably vary above 5 per cent. from day to day, though seldom getting far away from what we might call the absolute normal. The following results were obtained by us from examining at one test a number of supposedly normal persons against tubercle bacilli and staphylococci.

Opsonic Counts in Test of Twenty-one Normal Sera with the Same Stock Staphylococcus Culture.

1.....	4.13	8.....	3.82	15.....	9.09
2.....	2.93	9.....	3.95	16.....	5.17
3.....	2.78	10.....	3.98	17.....	4.04
4.....	4.37	11.....	4.27	18.....	3.82
5.....	3.58	12.....	3.69	19.....	4.00
6.....	2.90	13.....	3.80	20.....	3.79
7.....	3.56	14.....	3.59	21.....	3.44

Opsonic Index in Eighteen Normal Cases with Tubercle Bacilli.

Case.	Average Number Bacilli.	Opsonic Index.
1.....	2.46	1.00
2.....	3.20	1.3
3.....	2.90	1.14
4.....	2.66	1.08
5.....	2.75	1.12
6.....	2.30	.94
7.....	2.40	.98
8.....	1.88	.76
9.....	1.73	.70
10.....	2.05	.83
11.....	2.21	.90
12.....	2.86	1.17
13.....	2.81	1.14
14.....	2.79	1.14
15.....	3.34	1.32
16.....	2.96	1.02
17.....	2.16	.88
18.....	3.12	1.27

The Influence Upon the Opsonic Test of the Specific Differences Between Strains of a Single Species—The general practice in laboratories is to use stock cultures of tubercle bacilli, staphylococci, and other bacteria for the opsonic tests. To obtain a culture from a case may be at first impossible and, if successful, causes a delay of at least one or two

days. The culture when obtained may also, as is frequently the case with pneumococci and streptococci, fail to opsonize.

These and other reasons tend to establish the use of laboratory stock cultures, and yet we must acknowledge that when we test the amount of opsonins by both the stock and fresh cultures, a marked difference sometimes develops, so that the index may vary at least 50 per cent. This factor of individual specificity must therefore be taken into account in our interpretation of the accuracy of an opsonic test.

Results from Using Two Different Strains of Staphylococci.

Staphylococcus cultures.

Serum.	First Day's Test.		Second Day's Test.	
	Strain A.	Strain B.	Strain A.	Strain B.
Case 1.....	8.25	4.09	3.00	2.39
Case 2.....	6.65	5.92	2.05	1.12

The Leucocytes to be Employed—To many it seems a matter of indifference whether one person's leucocytes or another's is used, but our experiences have shown that the leucocytes from different persons not only vary in their activity, but also in their selective action, and that the index is not the same when obtained with one person's leucocytes as with another's.

TABLE.

Tests of same emulsion of staphylococci with different leucocytes.

Specimen of Blood from	Leucocytes of B. Bacteria per Cell.	Leucocytes R. Bacteria per Cell.
Gibson	2.8	2.9
Bolduan.....	3.0	3.4
Jurist.....	3.0	3.4
Greenwald	2.6	4.4

The Opsonic Variation During Treatment by Inoculations—Wright lays stress on the considerable uniformity of the degree and persistence of development of opsonins after inoculation. We have found in a small

percentage of cases typical increases and decreases, but in the majority of those inoculated there has been great irregularity. Our experience has, of course, been extremely limited when compared to that of Wright.

The following chart for three staphylococcus cases illustrates this:

3.0

2.5

2.0

1.5

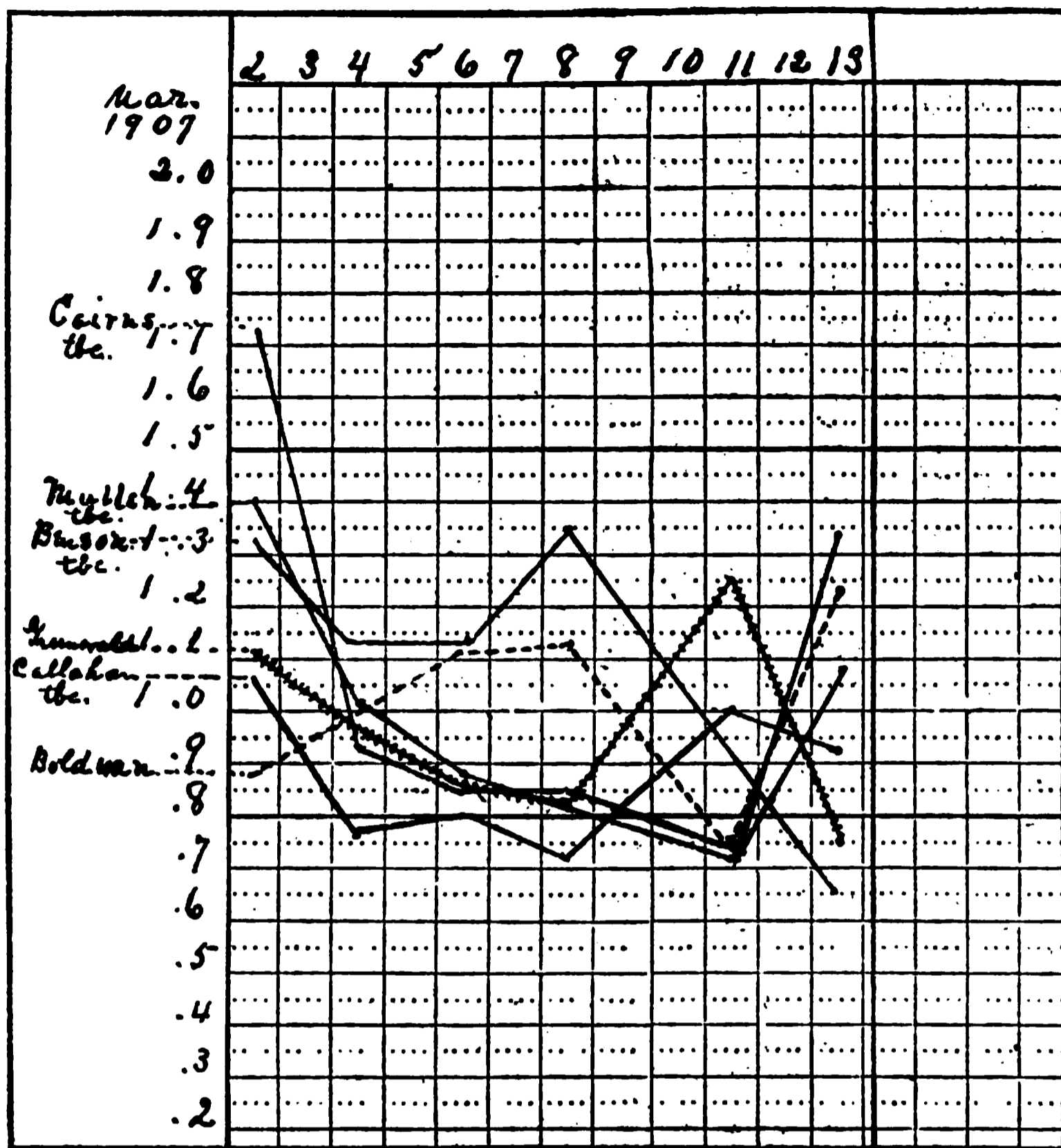
1.0

.5

.0

x = injection of vaccine.

The Variation in the Amount of Opsonins in Supposedly Healthy Persons—It has already been noted that in getting our measure we test a number of persons and exclude the blood of those which vary greatly from the average. The fact that it is necessary to exclude the blood of some shows that variation occurs. We are so in the habit of seeing the normal blood placed at unity because it is each day the measure of comparison that even investigators are apt to think of the indices of normal persons as being unchanged from day to day. This is not the fact. A glance at the next chart, in which three cases of tuberculosis are charted together with two normal persons, shows that the variation is only slightly greater in infected than in normal cases. If one normal person is charted against another for several weeks marked differences will usually appear. The indices of the twenty-one normal cases tested against staphylococci and the eighteen against tubercle bacilli, recorded on page 13, illustrate this variation in the amount of opsonins in normal blood.



Opsonic curves in normal and infected cases.

Dotted lines, normal cases. Continuous lines, tubercle cases (Phthisis).

The Opsonic Index Cannot Be Known at the Time the Treatment Is Given—Most of those who have not carried out inoculations under the guide of the opsonic tests think that the vaccinator is guided at the moment of injection by his knowledge of the opsonic power of the blood at the time. A moment's thought reveals that this is an absolute impossibility. In fact, except under very unusual conditions, it is impossible to have the test of the opsonic power reported within twenty-four hours, and in the treatment of the poor in out-patient practice longer intervals usually elapse, so that the treatment is given on a test made either the day before, or more often on from three to seven days before. As you will see by the three curves on page 15, which are quite as

uniform as the average, it is impossible even to guess what the index is at any moment by looking at the indices taken from one to seven days previously. This impossibility of knowing the opsonic power at the moment of inoculation is in itself a very serious difficulty, and should not be overlooked, as it practically is.

The Nature of Opsonins—The work in our laboratories has been, during the past six months, largely devoted to acquiring technic and studying the results of inoculation. Only a little research work has been done on the nature of opsonins.

Wright and Neufeld, in their original experiments, differed as to the effect of heat on opsonins. Wright claimed they were thermo-labile and Neufeld that they were the opposite. Further investigation has shown that the opsonin in those not immunized is largely thermo-labile, and those developed because of immunization are resistant. Muir and Martin believe from their experiments that the thermo-labile opsonin of normal serum and the thermo-stable opsonin are two entirely distinct classes of substances. The thermo-stable substance is of the nature of a true antibody and possesses the comparatively specific qualities of antibodies in general. Powerful complement absorbers have no effect on the thermo-stable opsonin, but do remove almost completely the thermo-labile opsonin.

Emulsions of other than the organisms used in immunization do not absorb a large percentage of the immune opsonin, but do of the complement opsonin.

We have carried out absorption experiments with staphylococci, colon and tubercle bacilli. Our results were similar to those of Muir and Martin. With more closely allied bacteria such as colon and dysentery bacilli, there would be, in all probability, more group absorption.

Comparison Between Opsonins and Bactericidal Substance in the Serum—We have made comparative tests between the opsonic and bactericidal power of the blood in typhoid infection and found that they did not run parallel. The frequent rapid increase in opsonic power within twelve hours of an injection of bacteria is striking and very different from the development of bactericidal strength.

Treatment—The number of cases which we have treated with vaccines has been comparatively few. We began with the treatment of

twelve cases of tuberculosis, of which half were tuberculosis of glands or joints and half were incipient lung cases. The opsonic index responded so irregularly to the injections that we were unable to derive help from it in treatment and after four weeks we discontinued taking the index, but continued the vaccine treatment according to the system in use at Saranac Lake and elsewhere.

Nine cases of staphylococcus furunculosis were treated apparently with benefit. In three of the nine, before inoculation were begun, the index was above the normal, in the others it was slightly or considerably below normal. After injection the opsonic power of the blood always rose above the normal, but sometimes only for a few hours. When the index was taken every day, it was so variable as to be of little help, and now we treat the cases without taking the index.

A number of cases of furunculosis came to our attention, during the time when the others were being treated, who refused to be injected. These all made good recoveries, but none of these cases were as severe as several of those receiving inoculation. Our belief is that the vaccines aided in the recoveries of those inoculated.

A number of mixed infections of the sinuses of the head were treated. In these cases great care was taken to thoroughly cleanse the cavities before taking the cultures. Although at first some of these cases seemed to be improved, by giving vaccines prepared from their own cultures, yet in the end only one out of eight showed definite improvement, and the vaccine injections were, therefore, replaced by operation and the usual treatment.

A number of cases of vaginitis in children, due to gonococcus, were inoculated with the gonococcus vaccine. For every case so treated a control was cared for in the ordinary way, and there was no marked difference in the behavior of the two series of cases. A detailed account of these cases is found in the next paper. In a case of gonorrheal arthritis benefit seemed to follow the injections.

Two cases having chronic sinuses leading to diseased bone were treated by the vaccines without benefit. Both were infected with more than one organism. In one of these there were three different organisms, two of these, which were unidentified bacilli, had remarkable in-

dices, one averaging above six and the other above three during several weeks in which the tests were made.

Three cases of acne were treated. Two of these were quite severe and were apparently benefited. They had previously been resistant to the ordinary treatment.

The few cases which we have studied have impressed upon us the opinion that while the vaccines have a restricted field in therapeutics, it is greater than we believed possible before the reports of Wright were published.

Those which appeared to receive the greatest benefit were localized infections of the subcutaneous or deeper tissues. Inflammations of the mucous membranes and generalized infections were not apparently benefited. Our experience in the vaccine treatment is, however, so limited, when compared to that of Wright and his followers, that we feel we are hardly justified in making conclusions even as definite as above.

The opsonic index does not seem to us to be an adequate guide to regulate the use of vaccines.

Other methods of estimating the opsonic power of the blood have been tried, but none of these have seemed to us to be capable of guiding the injections of vaccines in the routine treatment of disease.

VACCINE TREATMENT OF THREE CASES OF GONORRHEAL VAGINITIS IN CHILDREN, WITH A STUDY OF THEIR OPSONIC INDICES.

By ADELE OPPENHEIMER and HARRIET L. WILCOX.

Stimulated by the work of Wright and his school, we studied the curative value of gonococcus inoculations in gonorrheal vaginitis in children, and made determinations of the opsonic content of the blood serum during the progress of these cases. We did not, however, use the opsonic index as a criterion for therapeutic purposes, but gave injections at regular intervals of four to eight days irrespective of the opsonic curve. The hospital in which these patients were kept is a considerable distance from the laboratory and not conveniently reached. For this reason daily opsonic examinations were out of the question, and we had to content ourselves with trying to determine the opsonic index, first, immediately before the injection; second, twenty-four hours later, and third, three days later still, and thus follow as best we could the effect produced on the blood serum by the vaccine.

As is well known, in institutions where children are collected together, gonorrheal vaginitis among the female children is relatively common, occurring sporadically or in epidemics, even where, as far as can be discovered, every precaution is taken. For this reason all such cases are rigidly excluded from certain hospitals; but in the public hospitals of a city such exclusion cannot be practiced, and especially not in a hospital for contagious diseases.

In our hospital for contagious diseases careful vaginal smears are made of every female child before admission and where vaginal discharge exists, isolation is enforced. Despite this precaution cases crop out occasionally where the infection has apparently taken place in the hospital. In such instances the child, even when she is well of the disease for which she was admitted, cannot be discharged from the hospital until she is also cured (?) of the vaginitis. Everyone who has studied the question knows that some of these cases of vaginitis are of very long duration; for example, Number One of our series had a profuse vaginal discharge for some five weeks before the vaccine treatment was undertaken, certainly a great additional expense to the city. There

was then a practical, as well as a humane and scientific reason for trying Wright's method of vaccination, in the hope of securing a more rapid recovery from the disease than when local treatment alone is given.

One great difficulty which we encountered in the technique was in obtaining a homogeneous emulsion of the gonococcus. The first strain which we attempted to use was very unsatisfactory on account of its clumping. To obviate these clumps, a small amount of sterile sand was added to the emulsions of the organisms, made with different strengths of salt solution, namely, 0.8 per cent., 1 per cent., 1.2 per cent. and 2 per cent., and the mixtures then shaken steadily for five minutes; the sand was thereupon allowed to settle out and the supernatant fluid was examined microscopically, only to find numerous small clumps still present. Different grades of filter paper were then used for filtering a heavy emulsion, but the results were not successful, as the filtrate in each case contained more or less clumps. A second strain of the gonococcus was obtained, which gave a very good emulsion when grown on ascitic agar neutral to phenolphthalein, the growth being much lighter on this medium than on ascitic agar neutral to litmus. Another culture of the gonococcus which gave a very good suspension was isolated by Dr. von Sholly from the vagina of a scarlet fever patient. The child had had a profuse vaginal discharge for four weeks or more, and it was decided to give her vaccine treatment with the autogenous organism and to test the blood for the opsonic content with the same strain.

The technique for measuring the opsonic index used was that devised by Wright. An emulsion of living organisms from a twenty-four-hour culture on ascitic agar was made in sterile 0.8 per cent. NaCl solution and allowed to stand for an hour or two before using, so that any clumps present might settle out, leaving a good homogeneous suspension. The blood for the leucocytes was collected in sterile 1 per cent. sodium-citrate-salt-solution and centrifugalized for forty-five minutes at medium speed. The normal sera were obtained from three normal adults on the same day as those of the patients and were tested at the same time, and the average phagocytic power of leucocytes under the influence of these three normal sera respectively served as unity. The number of bacteria in each of one hundred polymorphonuclear leucocytes was counted for each serum on each such day, in the belief that

this would give us a true indication as to whether the number of organisms in the phagocytes was large or small. The tubes containing the mixture of serum, leucocytes and bacteria were incubated for fifteen minutes and were rotated, one, two, or three times during this period to insure continued mixing of the contents of the tube.

The vaccine was made from a twenty-four-hour culture on ascitic agar, with sterile normal salt solution, and was standardized for one million organisms per cubic centimeter. This emulsion was heated to 65 degrees C. for one hour; cultures were made after half an hour's and after one hour's heating to test the sterility and finally lysol in the proportion of 1-400 was added as a preservative. *After forty-eight hours an abundant growth was found on the ascitic agar inoculated from the emulsion heated to 65 degrees C. for half an hour*, while that inoculated with the emulsion after one hour's heating was sterile.

Nine cases were chosen from which blood was collected and tested. Cases 1, 2 and 3 were children with gonorrheal vaginitis, who received vaccine treatment, together with local treatment part of the time; cases 4, 5 and 6 were also children with gonorrheal vaginitis, but received local treatment only; cases 7, 8 and 9 were normal adults. Case 1 received two inoculations of the vaccine and the blood was tested twice for the opsonic index before it was decided to treat two other children in the same way, and to control these with three unvaccinated children also having gonorrheal vaginitis, *e. g.*, cases 4, 5 and 6. All three cases, 1, 2 and 3, received injections of vaccine made from the organism isolated from case 1, and the serum from each of the nine cases was tested for its opsonic content with this same strain of gonococcus.

On January 3, 1907, a specimen of blood was taken from Case 1 and tested with the autogenous organism; the opsonic index was found to be normal. Two days later the patient was inoculated with one cubic centimeter of the vaccine. On January 7, two days after the first injection, the opsonic index was 1.2 and continued to rise until twenty-four hours after the second injection, when there was a marked fall in the index to .5 below normal. From this time on, the blood was regularly tested, with one exception, on the day it was obtained.

On January 14 Case 2 received the first injection and Case 1 the third. On January 21 Cases 1 and 2 were again inoculated and Case 3

received the first injection. The three cases were then regularly inoculated once a week with one cubic centimeter of vaccine, standardized either for one million or two million organisms per cubic centimeter as shown on the curves.

The blood from these three cases was collected and tested at the same time and under the same conditions as that of the three controls (Cases 4, 5 and 6), and the three normals (Cases 7, 8 and 9). Two of the controls were discharged from the hospital one week after the test was begun, so it was impossible to obtain their blood for further examination, therefore, two other children having gonorrheal vaginitis were substituted as controls.

Histories.

Case 1, G. J.—Six and one-half years old. Admitted to Kingston Avenue Hospital on fifth day of scarlet fever. Vaginal smear negative on admission. In her eighth week at the hospital, vaginal discharge noted and smear showed pus cells with gonococcus. Vaginal irrigations with boric acid solution followed by 2 per cent. silver nitrate solution given twice daily. Vaginal discharge persisted in spite of treatment. Vaccination begun about five weeks after discharge was first noted. Improvement after first injection for almost a week; irrigations were stopped when the vaccine treatment was begun, but had to be resumed after two weeks (January 19), on account of profuse discharge. Discharge gradually decreased under vaccine and local treatment, and finally ceased so that the silver nitrate irrigation was stopped (February 4), about one month after vaccine treatment had been started. On February 9 patient was sent home as cured.

Case 2, G. B.—Age six. Admitted to Kingston Avenue Hospital with scarlet fever. Vaginal smear on admission was negative. Twelve days after admission, vaginal discharge was noted with pus cells, subsequently gonococci found. Local treatment as in Case 1, except for two weeks after first vaccination (January 14). Vaccination with same organism as in Case 1. Smear was negative February 16 and no vaginal discharge after February 19. Treatment ceased the nineteenth. Patient was discharged on February 24.

Case 3, S. S.—Four years old. Admitted to Kingston Avenue Hospital with scarlet fever. Smear was negative on admission. Vaginitis

developed five weeks after admission. Received first inoculation on January 21. The discharge gradually diminished. Received local treatment, except for about ten days after first inoculation. Still had vaginal discharge when vaccine treatment was stopped February 19. Local treatment was continued until March 5, when vaginal discharge had completely ceased. Patient sent home on March 12 cured (?).

Cases 4, 5, 6—Diagnosis was scarlet fever. Smears were negative on admission. Vaginal discharge developed one month, two and three weeks respectively, after admission. One received irrigation of boric acid and silver nitrate, two received potassium permanganate irrigations. Two had profuse vaginal discharge with abundant organisms, while blood was being tested. One, Case 6, had very little discharge with occasional organism in pus cells, being practically well, as far as gross discharge was concerned, before we started testing.

Cases 7, 8, 9—Were normal adults, workers in the laboratory. The blood from these cases was collected and tested on the same day as the above with the exception of January 22, when it was not possible to obtain blood from Cases 8 and 6.

Conclusions.

Our work has been carried on upon so few cases that we can give but tentative conclusions.

(1) Every one of the six patients, both those with and those without vaccine treatment, was discharged from the hospital as cured within twelve weeks from the beginning of the gonorrheal vaginal discharge. The patient who suffered longest from the gonorrheal vaginitis (Case 3), had both forms of treatment for a while, but, after all, she was only ill of gonorrheal vaginitis six weeks after the first and two weeks after the last inoculation. Moreover, Case 4, with only local treatment, showed a discharge for about ten weeks. Of the other two patients who received both kinds of treatment, and the injection as well as the irrigation to the last, Case 1 was cured (?) in about four weeks and Case 2 in about five weeks after the first injection.

On the other hand Cases 5 and 6, who were given local irrigation only, were cured (?) in about five and one-half weeks and in about three weeks respectively.

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In comparing these very few cases of gonorrheal vaginitis in children, namely, the three with vaccine treatment, in addition to local treatment, and the three with only irrigation treatment, we see that the vaccine treatment did not shorten the duration of the disease markedly, if at all.

(2) Nothing in the general or local conditions of the cases explained the opsonic indices on the various days on which they were estimated, but

(3) There were greater fluctuations in the opsonic curves of the vaginitis cases than in the normal, especially above unity rather than below. The apparent exception in Case 6 will be made clear by reading the history of the case, which shows Case 6 to have been practically normal most of the time.

(4) After each inoculation, the opsonic index apparently rose or continued to rise, excepting only in Case 3, between January 28 and February 4.

(5) We did not observe any negative phase (which, according to Wright, immediately follows a bacterial inoculation), following the injection, but it should be remembered that the blood was not tested until twenty-four hours after the inoculation. In this experience, we agree, however, with those in our laboratory who have worked with microorganisms other than the gonococcus, and who tested the opsonic power shortly after inoculation with bacterial vaccines. (See the preceding paper by Park and Biggs).

(6) Judging by the curves of the three cases of vaginitis which were not vaccinated, auto-inoculation is hard to avoid, and therefore the opsonic curve does not promise to be of value in suggesting the times for injection.

(7) Judging by the opsonic curves of the normal cases, what Wright would call a low opsonic index is not of diagnostic value; on the other hand a comparative study of the curves leads one to suspect that an unusually high index might serve as a diagnostic hint.

(8) Finally, by the way, our results with normal sera and gonococcus confirm those fluctuations reported by Bolduan* of the Research

* Bolduan, Long Island Med. Journal, Vol. I., 1907.

Laboratory with regard to normal sera and tubercle, staphylococcus and streptococcus, namely :

(a) The opsonic power often differs very widely in different normal individuals. See curves of Cases VII., VIII. and IX.

(b) From day to day the fluctuations of the opsonic index of one and the same normal serum are great and that without any apparent cause. See curves of Cases VII., VIII. and IX.

(c) There is an enormous difference in the phagocytic activity of the different leucocytes under the influence of the same serum in the same tube, as exhibited by the difference in the number of ingested bacteria. For instance, on one slide, taken at random, the average number of gonococci in a polymorphonuclear leucocyte was 2.86; each of ten polymorphonuclear leucocytes contained 10 or more gonococci, and each of forty-three such leucocytes contained none at all.

We wish to thank Dr. Binford Throne, of the Kingston Avenue Hospital, for his generous assistance there.

Supplement.

After the work described above was completed, we had the opportunity to study comparatively, but for less than a month only, three additional cases of gonorrheal vaginitis in children, namely, Case 10, given vaccine treatment only, Case 11, given irrigation treatment only, and Case 12, left untreated.

The method pursued was exactly like that described above, except that in obtaining the leucocytes, we centrifugalized, not forty-five minutes at moderate speed, but fifteen minutes at high speed. In our previous investigation we had discovered that such a difference in the manner of centrifugalization made no difference whatsoever in the leucocytes. On most days the normal sera were obtained from one normal adult, and from two children convalescent from scarlet fever.

The vaccine was made from the organism obtained from Case 10 and standardized for two million organisms per cubic centimeter. All the sera were tested for the opsonic content with the organism isolated from Case 1. The serum from the vaccinated patient and the two control sera (from the irrigated and untreated cases respectively), and the normal sera were collected at one time under the same conditions.

On March 21 and 28 and on April 4 Case 10 was inoculated with the autogenous organism. On each of these days before the injection and each time on the day following the injection, a specimen of blood was taken from Case 10 and was then tested for the opsonic index. Also two or three days before the inoculation the serum was collected for testing.

On these self-same days (namely, on March 19, 21 and 22*, 25, 28 and 29, and on April 1, 4 and 5), blood was collected for estimating opsonic content from the control Cases 11 and 12 and from four normal Cases 13 and 17, and 15 and 16.

In addition, between March 8 and April 19 the opsonic indices of Cases 10 and 11 (vaccinated and irrigated respectively), and of the normal Cases 13, 14, 15, 17 were obtained a few times as indicated in the curves. The normals were changed when, for one reason or another, it was impossible to use their blood for further examination.

Histories.

All the cases, excepting only Case 13, were children admitted to the Willard Parker Hospital with scarlet fever.

Case 10, R. G.—Three years and four months old. Gonorrheal vaginitis on admission. Vaccine treatment was begun nineteen days after admission and given until this investigation was discontinued, that is, the vaccine treatment was given for about two weeks.

Case 11, F. P.—Age four years. Gonorrheal vaginitis on admission. Vaginal irrigations with potassium permanganate given twice daily throughout her stay in the hospital. While her discharge was somewhat less, her smears still showed gonococci when she left the hospital.

Case 12, H. M.—Six years old. Gonorrheal vaginitis on admission. Gonococci still present up to the time that we stopped taking her blood.

Case 13—Normal adult, worker in the laboratory. The blood from this case was collected and tested, as normal to gonococcus, on the same days as the above with the exception of March 8 and 11, when it had not yet been decided to use this blood.

* Cases 11 and 12 not examined on March 22d.

Case 14, J. M.—Girl, age three years and two months. The blood from this case was collected as normal to gonococcus, on March 8 and 11.

Case 15, L. F.—Girl of four years. The blood from this case was collected and tested, as normal, from March 8 to 25, inclusive.

Case 16, W. D.—Boy, age four years. The blood collected, as normal, from March 28 to April 5, inclusive.

Case 17, S. T.—Boy, four years old. The blood collected, as normal, from March 15 to April 5, inclusive.

Summary.

(1) No one of the three patients had been cured of gonorrheal vaginitis up to the time when the investigation had to be discontinued, so no further light was thrown on the value of the vaccine treatment.

(2) All the other conclusions of this study, namely (2) to (8), inclusive, on page 25, were corroborated by this supplementary work.

OPSONIC INDEX CHART

NAME Goda Judman DIAGNOSIS Vaginitis CASE NO. 1NAME Grace Berma DIAGNOSIS Vaginitis CASE NO. II (2)

OPSONIC INDEX CHART

NAME..... *Rutherford, Sadie* .. . DIAGNOSIS..... *Fajinilla* .. . CASE NO. *III* .. .

NAME..... *Charles T. Jones* .. . DIAGNOSIS..... *Fajinilla* .. . CASE NO. *IV* .. .

NAME Mordak, Sula DIAGNOSIS Typhoid CASE NO. I

OPSONIC INDEX CHART

NAME Oxenhandler, Mollie DIAGNOSIS Typhoid CASE NO. VI

THE IMPORTANCE OF ICE IN THE PRODUCTION OF TYPHOID FEVER.*

WILLIAM H. PARK, M. D.

We may endeavor to settle this question directly by determining whether epidemics or scattered cases of typhoid fever have been traced to ice, or, failing in this, we may try to estimate the probability of such infection by learning the duration of life of the typhoid bacillus after freezing.

The total number of instances of typhoid fever which have been directly traced to ice infection are remarkably few. I have been unable to trace more than three. One was in France, where a group of officers placed ice made from water polluted by a sewer in their wine and afterward developed typhoid fever, while those of the same company not using ice escaped. A second case was in a small epidemic brought to my notice which occurred in those who used ice from a pond. It was found that water directly infected with typhoid feces had flowed over its frozen surface had been congealed there. A third case followed the use of ice from the St. Lawrence river, gathered at a point at which sewage pollution was marked. In this instance bacilli were isolated from the ice which were believed to be typhoid. If typhoid fever is communicated through ice, except under exceptional conditions, it is remarkable that so few cases are traced to it.

The fact that freezing kills a large percentage of typhoid bacilli makes it indeed possible to conceive that ice from moderately infected water contains so few living typhoid bacilli that only the exceptional person here and there becomes infected, and so the source of the infection remains undetected.

If this be true and scattered cases occur, there should be at least some increase on some or every year in March, April and May in such a city as New York, where four-fifths of all the ice consumed is from the Hudson River, which is known to be contaminated with typhoid bacilli. The people of New York use ice very freely and most of them

* Read in the Section on Hygiene and Sanitary Science of the American Medical Association, at the Fifty-eighth Annual Session, held at Atlantic City, June, 1907.

put it directly in their water or place their vegetables on it. The new ice from the Hudson River is gathered in January or February and stored on top of the left-over ice, and thus shipments to the city are immediately begun. It is an established fact that typhoid bacilli in ice are most abundant during the days immediately after freezing. At the end of two months less than 1 per cent. of the original number survive.

If Hudson River ice produced an appreciable amount of typhoid fever, this would then be noticeable in March and in April and perhaps in May.

When we examine the records for the past ten years we find no increase of typhoid fever in Greater New York during those months, with the one exception of the present year, when we had in the Borough of Manhattan a sharp outbreak lasting four weeks. This outbreak did not occur at all in Brooklyn. As the people of Brooklyn drank different water, but received ice from the same places of the Hudson River as those of Manhattan, this directed attention to the water or milk rather than the ice. Examination of the Croton watershed at the time showed that a small epidemic of typhoid existed there and that pollution of the water was probable. This suggested still more strongly that the water and not the ice was the cause of the typhoid infection.

It happened that most of the cases occurred in those living in the section of the upper West Side, where only well-to-do people live. An investigation showed that the majority of the infected had used only artificial ice and several had used no ice in their water at all.

Let us now turn our attention to the life of the typhoid bacillus in ice in laboratory experiments. The first important investigation was that of Prudden, who showed that typhoid bacilli might live for three months or longer in ice. This experiment is frequently wrongly interpreted, as when a recent writer states: "It has been amply demonstrated that the germs of typhoid fever are not killed by freezing and that they have been known to live in ice for long periods of time."

It is true that in Dr. Prudden's experiment a few typhoid bacilli remained alive for three months, when the experiment was terminated, but these were but a small fraction of 1 per cent. of the original number. Following Prudden's experiment, Sedgwick and Winslow in Boston,

and I in New York City carried on independently. These led to the same conclusions. A final experiment of mine in which twenty-one different strains of recent isolation, were subjected to the test is given below.

Life of Twenty-one Strains of Typhoid Bacilli

	Average Number of Bacilli in Ice.
Before freezing.....	2,560,410
Frozen three days.....	1,089,470
Frozen seven days.....	361,136
Frozen fourteen days.....	203,300
Frozen twenty-one days.....	10,280
Frozen twenty-eight days.....	4,540
Frozen five weeks.....	2,950
Frozen seven weeks.....	2,302
Frozen nine weeks.....	127
Frozen sixteen weeks.....	107
Frozen twenty-two weeks.....	0

In these experiments twenty-one different flasks were inoculated each with a different strain of typhoid. A little of the feces rich in typhoid was directly added to the water in each flask was then pipetted into thirty tubes which were placed in a cold storage room in which the temperature was from 20 to 28 degrees F. At first tubes were removed once a week, later once a week. The object of using so many tubes was because it has become evident that some cultures die more rapidly than others.

At the end of five weeks the water infected with typhoid was sterile, at the end of sixteen weeks only four strains were still living.

Two interesting investigations of Hudson River water have been completed, one by Dr. D. D. Jackson, for the city of New York, and the other by Dr. C. E. North, for a private laboratory.

There was noticed a considerable difference in the number of bacteria in the top, middle and bottom layers of water. This was since while water in freezing from above downwards, the bacteria are driven to the bottom.

itself, 75 per cent. of the solids and a fair proportion of bacteria being eliminated, yet this can not happen in the case of the snow blanket which becomes flooded by rain or by cutting holes through the ice. Here all impurities, such as dust and leaves which have fallen on the surface and dirt which may come from the water, remain with the bacteria which they carry. The bottom ice, being the most recently frozen, has had least destruction by freezing. Dr. Jackson's report gives us a rather unpleasant impression of Hudson River ice. He states that most of the samples either at the top or bottom show intestinal germs. The worst portions of the ice showed from 1 to 10 bacilli of the colon group per c.c. These were taken above or less than five miles below Albany. Some of this ice might aptly be termed solidified sewage.

The analysis of the twenty-five samples shows, as one would expect, that the surface of the ice just at Albany was badly contaminated, one c.c. of melted ice averaging about 15,000 bacteria for four samples. Only one of the twenty-one samples taken at different distances below Albany showed over 1,000 per c.c. A sample from top of a cake of ice obtained two miles below Albany showed only 14. The other twenty samples had on the average 105 per c.c.

Dr. North, in his investigation, examined the ice from forty spots between Hudson and Albany. He took samples from the top, middle and bottom of each cake and the water of the river. He has kindly permitted me to use some of his figures.

The river water in the forty specimens averaged 1,800 bacteria per c.c., the top ice 306, the bottom ice 36 and the middle ice 14. Only four specimens of top ice had over 500 bacteria per c.c., none of the specimens of middle or bottom ice.

Thirty-three of the specimens of water had over 500, and 23 over 1,000. Colon bacilli were obtained from but one specimen of the middle ice, two from the bottom ice and twelve from the top ice.

The great destruction by freezing is noticeable in these figures. Even the top ice soiled by the horses and men gathering it contained but 16 per cent. as many bacteria as the water from which it was contained. The bottom ice, the last to be frozen, had but 2 per cent. of those in the water.

Conclusions.

The danger from the use of ice produced from polluted water is always much less than the use of the water itself. Every week that the ice is stored the danger becomes less, so that at four weeks it has become as much purified from typhoid bacilli as if subjected to sand filtration. At the end of four months the danger becomes almost negligible, and at the end of six months quite so. The slight danger from freshly cut ice, as well as the natural desire not to put even sterilized frozen sewage in our water, suggests agreement with the report of Dr. Jackson, that portions of rivers greatly contaminated, such as the Hudson within three miles of Albany, should be condemned for harvesting ice for domestic purposes—such ice alone to be used where there is absolutely no contact with food.

STUDIES ON THE ETIOLOGY OF SCARLET FEVER.

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Assisted by

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Studies on scarlet fever have been carried on intermittently at the Research Laboratory of the Health Department for the last five years.

Our opportunities for the study of this second most common acute infectious disease have steadily increased in the past few years, and they are now exceptional. With an immense scarlet fever hospital at our door, full of patients, with an expert hospital staff ready to assist us in every way, practically the only drawbacks in our work are, first, the inability to obtain autopsies when death occurs during the active stages of the disease, and, second, the fact that so far as we know, man is the only animal susceptible to the disease. We are, therefore, unable to test our findings on experimental animals. The experiments on monkeys have as yet been too few for us to draw any conclusions as to their complete non-susceptibility.

Historical Review.

Notwithstanding the fact that this disease has been recognized as an entity since the time of Sydenham (1685), and that there is a lengthy bibliography in regard to it, we really possess comparatively little knowledge of its exact nature. This is due in great measure to the lack of careful observations and of minute records. Even from a clinical standpoint we are still in doubt in regard to many points.

(1) In the first place, we do not know definitely the distribution of the virus, and hence the chief source of infection.

Prior to 1893 the desquamating skin is most frequently mentioned as carrying the contagion of scarlet fever, though it must be noted that as early as 1883 Pohl Pinkus insisted on the disinfection of the mouth. During the nineties, the French authorities especially laid stress on the infectivity of the secretions of the mucous membrane of the throat, nose and ear, ignoring the desquamating skin. Since then, most writers have laid equal stress on both as possible modes of infection. In 1896 dis-

charges from post scarlatinal otorrhœ are mentioned as infectious, and hospital records bear witness to the truth of this observation. Later, urine, faeces, perspiration and even the breath, are spoken of. Since 1905 the general opinion seems to be that the primary desquamation and the secretions from the mucous membranes of the nasal and buccal cavities carry infection, but that the prolonged desquamation probably does not.

The great majority of so-called "return cases,"* according to recent statistics are thought to be due to infective material contained in the throat, nose and ear exudates. In order to be certain of this, the most careful oversight of discharged hospital cases should be carried on for some time after their return home, since a fresh attack of exudative inflammation in an apparently cleared-up case of recent scarlet fever is supposed to start up the development of remnants of quiescent scarlet fever virus.

Our discharged cases are now being kept sight of, as far as possible, with the idea of solving this problem.

(2) We do not know how long the virus may remain infective, when separated from the patient. The most extravagant tales are told of infection from exposed articles after twenty years' rest in a trunk.

(3) We do not know the exact period of incubation in this disease. The majority of authors think that the first symptoms may show any time from twenty-four hours to four weeks after exposure, while some recent good observers say that in all cases recorded as developing under four days, the possibility of an earlier exposure cannot be ruled out, and that the mean time of the different observers, twelve to fourteen days approximately, is probably the true period of incubation in this disease.

According to the work done on experimental infectivity, there seems to be but little doubt that the period of incubation may be as short as twenty-four hours. For instance, it has been shown that children can be infected with scarlet fever within twenty-four hours after inoculations subcutaneously with the mucus from the throat or mouth of scarlet fever patient.

In 1897, J. W. Stickler, of Newark, claimed to have produced genuine scarlet fever in ten children by such inoculations. The mucus was

* By "return cases" we mean cases which in all probability have become infected from discharged hospital cases.

first treated with carbolic acid, 1/600th part, before inoculation. All the children developed the scarlet fever symptoms beginning in less than twenty-four hours.

Leube (Rep. Archives of Pediatrics, October, 1905), injured his finger during an autopsy on a severe scarlet fever case. On the seventh day the wound pained, on the tenth day there was angina and malaise and later a rash appeared.

(4) It has been shown by this experimental work on infectivity that it is difficult to produce scarlet fever in animals. The inoculations by which Stickler produced scarlet fever in children, in white rabbits gave only local effects. Grünbaum, in 1904, was successful in producing a scarlatinal tonsillitis in a chimpanzee by rubbing the throat of the chimpanzee with swab from the patient's throat. On the fourth day a doubtful rash developed; on the fifth day an exudate on the tonsils appeared, which soon covered both tonsils. The sixth day the rash spread mostly over the abdomen but was not scarlatiniform in appearance. "Streptococcus conglomeratus" was isolated from the throat of the chimpanzee. The monkey's own blood agglutinated it. Grünbaum tried many other inoculation experiments. He found the streptococcus isolated from scarlet fever cases fatal to rabbits, but producing only suppuration in monkeys.

A disease resembling scarlet fever has at times been reported in cows. In Great Britain from 1882 to 1892, inclusive, seventeen epidemics (varying from 5 to 635 cases), were traced directly to a disease in the cows supplying the families infected. In eight of the cases mention is made of an eruption on the udder. In 1885, during the epidemic at Marylebone, due to disease in the Hendon cows, Klein isolated a streptococcus from these animals, and after comparing it with streptococci isolated by him from scarlet fever cases (1886-7), he believed the organisms were identical and the disease was scarlet fever.

Dr. Park investigated an epidemic of scarlet fever at Ossining several years ago, which occurred in the practice of Dr. Barnum, and considered that it was undoubtedly caused by the use of milk from a septic cow. The cow had recently calved, had a high temperature and great quantities of streptococci were found in the milk and in the

vaginal discharge. There were over thirty cases of scarlet fever in the two boys' schools which received the milk. The cases showed symptoms forty-eight hours after drinking the milk. None of the day scholars, who did not drink milk, developed the disease, and there was no scarlet fever in the town. Dr. Park reports that while the individual cases appeared as mild scarlet fever, the outbreak on the whole seemed somewhat different from ordinary scarlet fever. There was only one case which contracted scarlet fever from these boys.

(5) Though much has been written about the rôle of the streptococcus in scarlet fever, and, while many still hold that the streptococcus pyogenes, or a closely related streptococcus, is the real cause, there is no positive proof that this is so. The descriptions given of the organisms isolated and studied are too indefinite to allow satisfactory corroboration, and, therefore, claims for a specific variety of streptococcus amount to little. It is impossible to draw any conclusions from the reports of the earliest writers, because of their inadequate knowledge of bacteriologic technic. The first clear description of a streptococcus isolated from scarlet fever was given by Klein in 1886. He isolated the organism from the blood of five out of eleven early cases of the disease. He lays stress upon the irregular morphology and conglomerate growth (in broth), in distinguishing from other streptococci.

Baginsky, Summerfield, Ruediger, Jochmann, etc., found "streptococcus" in the blood of living cases of scarlet fever. Among the workers who have continued the study of the subject, the following have expressed the belief that the streptococcus is the cause of scarlet fever—Berge, Sverenson, Brunner, H. Fischer, Baginsky, Summerfield, D'Espine, Moser, Marignac, Kurth, Gordon and Gabritschewsky—but the growing opinion seems to be that the streptococcus, though closely associated with scarlet fever is a secondary invader of the system and not the cause. Hektoen, Jochman, Besredka, Dopter, Mallory, Duval and Siegel express this opinion, while Ruediger, Cumpston and others are non-committal.

Jochmann (in 1904), examined the blood of 161 living cases of scarlet fever and found "streptococci" in 25 (about 15 per cent.). Out of 66 autopsy cases 50 showed the streptococcus in the blood (over 80 per cent.).

Of the recent workers, Gabritschewsky speaks most positively in favor of streptococcus as the cause of scarlet fever, stating that his experiments in the use of streptococcus vaccine furnish new proof in favor of the streptococcus origin of this disease. In one series of 120 children vaccinated with streptococcus emulsion, he states that 13 per cent. showed symptoms of scarlet fever.

In regard to the variety of streptococcus isolated from scarlet fever by the various authors there is much diversity of opinion. Many simply speak of isolating "the streptococcus." Some consider that the streptococcus of scarlet fever is identical with streptococcus pyogenes.

Class, in 1889, isolated a diplococcus which he considered the specific cause of scarlet fever, but no other investigators agree with him in this.

In 1891 Kurth described the "streptococcus conglomeratus" as the cause of scarlet fever. Klein states that Kurth's description of this streptococcus agrees with his own, and that, therefore, the two organisms are probably the same species. Gordon also described a similar streptococcus.

Baginsky, 1900, states that he found the same streptococcus in the blood and bone marrow of 42 scarlet fever autopsies, but that it could not be distinguished from other varieties of streptococcus.

We cannot find any mention, in Gabritschewsky's articles of 1906 and 1907, of the variety of streptococcus used for the vaccine emulsions more than "streptococcus" from a scarlet fever patient; nor does he mention the culture generations used for the inoculation.

Marmorek recognizes no varieties of streptococcus. Besredka and Dopter (1904), state that no cultural or microscopic appearances or pathogenic actions seem to differentiate the streptococci. Agglutination is useless. They hoped for some help from the Bordet-Gengou reaction, but their hopes were not fulfilled. Foix and Mallein (1907), on the contrary, report positive results on using this method.

Moser says that the differences so far given between the streptococcus of scarlet fever and other streptococci are not sufficient to differentiate them, so other methods of differentiation must be sought. The streptococcus Moser used for some of his experiments showed short chains and diffuse cloudiness in bouillon.

Baginsky describes diplococci or short chains in the blood smears, long chains in the bouillon cultures, which returned to short chains after passage through animals.

From the above short resumé of the bibliography we see how impossible it is to arrive at a positive opinion in regard to a specific streptococcus in scarlet fever. The report of Anthony, working in this laboratory, on the hemolyzing qualities of streptococci in scarlet fever is given in another part of this volume.

(6) Only one of the many clinical symptoms of scarlet fever is said to be pathognomonic.

The *rash* is not characteristic, for we may obtain a perfectly typical scarlet eruption after inoculation of antitoxic or normal serum. *Desquamation* may occur after any erythema. Similar *throat symptoms* appear in ordinary tonsillitis.

The one symptom said by some to be pathognomonic is the enlargement of the papillae at the tip and sides of the tongue, and even this is sometimes so slight that it is difficult to make out, and it has been reported as occurring in other diseases.

The *complex of symptoms* is said to be characteristic in the majority of cases, but still many cases occur on the border line between serum rashes, measles and scarlet, and there has been much discussion over the "Fourth Disease of Duke," without arriving at a definite conclusion.

(7) Very little has been done on the minute pathology of scarlet fever, due largely to the reasons already given, that deaths occur so seldom during the height of the disease, and that it is so difficult to obtain autopsies. The only constant general change found is a hyperplasia of lymphatic tissue over the whole body.

Most attention has been given to the skin in the histologic study of this disease. Hebra and Kaposi describe hyperaemia. Klein was the first to make a careful study of the skin and there is no other report until that of Pearce in 1899.

He studied the changes in the skin on the different days of the disease, finding the infiltration with polymorphonuclear leucocytes most marked between the fifth and eighth days. He found the same epithelial changes in the pharynx, palate, and tonsil. In the tongue the leucocytes were most marked in the epithelium covering the papillae.

In 1904 Mallory studied the skin in living and autopsy cases, and described the bodies which he believes to be protozoa and the cause of scarlet fever, finding them in four autopsy cases. Two of these were of the severe toxic type and two were septic scarlet fever complicated with diphtheria. Rash was present in all four cases. Mallory found the skin from a number of living cases as well as from many autopsy cases negative. In his four cases he found the bodies in three situations, lying in vacuoles in the epithelial cells of the epidermis, to a less extent between these cells, and free in the lymph channels of the corium just beneath the epidermis. They were found usually in small clumps. The bodies were of two types, granular, or reticular, and radiate. Mallory gave the name "cyclasterion scarlatinale" to these bodies because of the radiate forms which he considered the more characteristic. Mallory's work has been little corroborated up to the present time.

In a study published in 1905, Duval agrees with Mallory that the bodies are protozoa and the cause of scarlet fever. He examined the serum from blisters, formed by ammonia, in 18 cases, and found the "Mallory bodies" in 5. He says also that he found them post-mortem in the skin of an infant whose blister serum showed them during life.

Field (1905), in our laboratory examined the skin from 20 living scarlet fever patients and from 10 autopsies, as well as from a number of controls, and came to the conclusion that most, if not all, of the bodies were degenerations of the cytoplasm of the host cells. He found none in living skin.

In 1905 R. L. Thompson, of St. Louis, states that he believes the Mallory bodies "to be protozoa and to have an etiological significance to scarlet fever," but that their significance cannot be made clear until they can be studied in cultivation.

In 1906 Leonard S. Dudgeon failed to find the bodies in sections from two cases which died on the fourth day with well-developed rash. He describes his technic as follows: Pieces of skin from a fatal case of toxic scarlet fever in an adult male. Death on fourth day of disease. Skin was fixed in Zenker and in salt formalin and stained with Leishman's stain (using a 0.5 per cent. solution of Leishman's powder in methyl alcohol).

Five of the recent editions of bacteriologies and pathologies mention the Mallory bodies in considering the possible cause of scarlet fever. McCollom in Osler's *Modern Medicine* (1907), says "the cause of scarlet fever is probably a protozoan as Mallory's work seems to demonstrate."

Several other workers have described protozoa-like bodies in scarlet fever, but their findings have not been confirmed, and are only added because of their historic interest.

Doehle in 1891 reported two forms of parasites found in the blood of five scarlet fever cases, one small flagellum-bearing organism, the other larger and granular.

In 1893 Pfeiffer states that he found a flagellum-bearing, freely motile amoeba in the blood serum of scarlet fever as late as the third day after the appearance of the eruption, and also found the same organism in variola, vaccine, zoster and measles.

Behla in 1893 describes small round protoplasm bodies, at times with flagella, in the blood of measles and scarlet fever.

J. Siegel in 1905 describes bodies in the skin and blood of scarlet fever, which he also found in small-pox, syphilis and foot and mouth diseases. He could not differentiate them in the various diseases, except in the skin by their position. His bodies are not like the Mallory bodies.

(8) Finally, more knowledge in regard to the geographic distribution of scarlet fever may be of assistance in studying its etiology.

The disease is very common in Germany, England and United States; common over the greater part of Europe (Italy one-half as many cases proportionately as England), Canada and South America; uncommon in Asia and Africa and in a few islands. It is rare in India, Madagascar and Japan. As late as 1903 two authorities pronounced it unknown in Japan. Ten cases were reported from Tokio Hospital in 1898 by M. J. Mayeda. (See *Pediatrics*, 1898, Vol. VI.).

McCollom, in Osler's *Modern Medicine*, mentions a zone of comparative immunity through part of the torrid zone. In comparing the geographical distribution of scarlet fever with erysipelas and puerperal fever as types of streptococcus diseases, we find the distribution of the

two latter more general than that of scarlet fever, but in the countries where they are most prevalent scarlet fever is also prevalent, and in India and Japan where they are rare, scarlet fever is rare.

Original Work—Having noted one or two small points which we considered advantageous in histologic technic while working on rabies, we decided to examine another series of scarlet fever cases, following, to a certain extent, the rabies technic in preparing our specimens.

Methods—Our technic was as follows: Very small pieces were fixed for a short time in Zenker's fluid. The important point here is the length of time in the fixative; from 5 to 10 hours we found best. From the Zenker fluid they were passed successively through iodine-alcohol, 70 per cent. and 96 per cent. alcohols, each for 24 hours, then for two hours in two absolute alcohols, cleared in cedar oil, imbedded in paraffin, and stained by various methods, chief among them being the following: Mallory's, iron-hematoxylin, safranin and "Lichtgrun," Unna's polychrom, Giemsa, safranin and gentian violet.

Specimens from two cases were prepared according to Levaditi's process for demonstrating spirochetes, and specimens from three cases were fixed in Fleming's solution and submitted to various stains.

Altogether sections of skin from 17 living cases, from 33 autopsies, and from 9 controls were examined. The following is a list of the cases examined, with some indication of the findings:

TABLE I.
Living Cases.

Case No.	Age.	Days Ill when Specimen was taken.	Intensity of Rash in General.	Characteristics of Rash at Time Specimen was Taken.	Microscopical Exam.	
					Bodies in Lymph Spaces.	Bodies in Epithelial Cells.
41	27	4	±	General erythema	+	—
42	2½	3	±	{ Light erythematation on neck, abdomen and chest	+	—
44	5	*21	+		—	—
59	2	3	+	Punctate—macular	±	—
60	21	7	+	{ Slightly faded. Primary desquamation on neck	±	—
61	21	6	+		+	+
62	16	4	+	Macular, beginning desquamation of neck and arms.....	+	±
63	9	3	+	Macular, well-developed.....	+	±
64	6	3	+	Macular	±	—
65	5	4	+	Macular	±	—
66	5	3	+	Slightly macular	+	±
70	4	6	+	Macular	—	±
71	8	4	+	General erythema.....	—	—
72	11	5	++	Macular and punctate, beginning vesicular	±	—
73	3	3	+	{ General intense erythema, beginning macular eryp. Desquamation on back	—	—
74	6	5	+		—	+
75	27	3	+	General punctate.....	—	±
				General macular; beginning desquamation on neck.....	—	±
				Light erythema slightly faded	+	±

* Case of recurrent scarlet fever with typical rash 21 days after first symptoms and rash which were typical. Desquamation occurred after each attack. Blood culture was negative.

TABLE II.
Autopsy Cases.

Case No.	Age	Days Ill.	Intensity of Rash.	Appearance of Skin Post Mortem, over Areas Cut for Sections.	Complications.	Sections Hours, P. M.	Culture	Microscopical Examination.	
								Bodies in Lymph Spaces.	Bodies in Epithelial Cells.
1	4½	3	+	Broncho-pneumonia, diphtheria following measles.	8½	+	+
2	2½	20	±	Diphtheria, mastoiditis....	33	-	+
11	2	2 (?)	+ Chin squamating..... abdomen, wrist and de-	Diphtheria.....	4	++	+
12	8	23	+	Thigh, desquamating; back, P. M. redness.....	Vaginitis, conjunctivitis; broncho-pneumonia; meningitis.....	4	-	-
13	3	4 (?)	+	Lower thigh, hemorrhagic spot; back, macular.....	Cervical adenitis.....	6	Blood, + { Pus, +	+	+
50	2	7	+	Back, P. M., redness.....	Broncho-pneumonia.....	7	Blood, + { Pus, +	±	±
53	5	6	+	Cervical adenitis; myocarditis.....	Same day.	Blood, + { Pus, +	-	-
206	3½	5	++ hemorrhagic	Ankle, hemorrhagic spot; wrist, spot; abdomen, eruption.....	14	Blood, +	++	+
217	3½	4	?	Side, P. M., redness, eruption; thigh, clear; back, P. M., redness, chest, eruption.....	5½	-	+	+
s.c. 24	2	13	++ hemorrhagic spots. (?)	Broncho-pneumonia, pericarditis; otitis media....	10	Blood, + { Pus, + { Blood, +	+	+
25	6	8	Back, P. M., redness.....	11	-	+	+
30	27	8	+	Chest, P. M., redness; chest desquamating.....	Nephritis, dermatitis, berpetiformis.....	23	Blood, +	++	+
34	6	(?)	Desquamating.	Desquamating.....	Erysipelas, nephritis, cancrum oris.....	9	-	-
37	21	(?)	(?)	?	?	2	Blood, -	+	-
39	2½	6	+	Diphtheria.....	2	Blood, -	-	-

TABLE III.
Control Cases.

Case No.	Age.	Days Ill.	Intensity of Rash.	Characteristics of Rash.	Complications.	Sections, p. hours.	Microscopical Examination.	
							Bodies in lymph space.	Bodies in epith. cells.
Measles, 31 living.....	10 months.	1	+	General macular, papular eruption, beginning to fade.....	Broncho-pneumonia.	±	±
Measles, 32 living.....	5 years.	1	+	General macular, papular, little faded on chest.....	—	—
Measles, 33 living.....	2 years.	1	+	Macular, papular eruption on extremities.....	—	+
Measles, 34 living.....	3 years.	2	+	Macular, papular eruption on face.....	±	—
Measles, 35 living.....	8 months.	2	+	Macular, papular eruption, beginning to fade.	—	—
Measles, 36 living.....	2 years.	3	+	Diffuse bright rash, fading on chest.....	±	—
550, autopsy.....	Deep burn.....	Septicaemia.	++	++
Diphtheria, autopsy, } 307.....	14 weeks.	7	..	Anti-toxin rash, scarlatina in form, persisting almost up to death	Broncho-pneumonia } intubation.....	15 mins. 18 hours 25 hours	—	—
Smallpox, autopsy, 207.	57 years.	5	++	Vesicular eruption over entire body; purulent on face, confluent on hands and legs	Slight systolic mitral murmur. Epileptic convulsions.	48 hours

In one of the living cases we found scattered groups of small reticular bodies in the lymph spaces, and in six of these an occasional similar small body within an epithelial cell.

In 27 of the autopsy cases, bodies have been found, in the lymph spaces in 20, in the epithelial cells in 15. In the majority of these, many bodies are seen, but they are practically all of the granular or reticular type. In several of the autopsy cases a few interesting groups of these finely reticular bodies were observed on the lymph spaces just beneath the epithelium. The forms are so characteristically amoeboid in outline and are so definitely grouped that they are quite suggestive of amoeboid protozoal forms. Through the kindness of Dr. Mallory, we have been able to study a number of sections from one of his cases, and we find that the reticular amoeboid bodies seen in our cases are quite similar to amoeboid forms in his sections. We found, however, no definite radiate or star-shaped bodies in any of our cases, and only occasionally did we see an indefinite rosette, while in the sections from Dr. Mallory's case both the rosettes and star-shaped bodies are very numerous and very definite. If these are specific organisms it seems strange that we have found comparative so few forms, and these the less characteristic ones, in all of our cases. It might, however, be accounted for by the fact that certain organisms (some protozoa), show only characteristic forms for a short time at definite stages of their life history, and produce tiny indefinite forms throughout the greater part of their existence. This is notably the case among certain amebida. One of us has an ameba under observation now which was isolated from the feces of a cat suffering from dysentery. This ameba in the adult form is a very small organism, with a definite nucleus. It changes quickly, however, by multiple budding into many tiny amebae, only a few of which show, with the best of staining, merely traces of nuclear material. The great majority of them appear as tiny reticular amoeboid forms showing no nuclei, very similar to the great majority of the forms found in scarlet fever. The finding, therefore, only of small apparently non-nuclear, reticular bodies, should not be considered strong evidence against such forms being protozoa.

On the other hand it is true that similar small reticular bodies may be formed as the result of degenerative changes in the tissues. We

found in one of our control cases many bodies apparently identical with the reticular bodies found in our scarlet fever skins.

This control skin was obtained from an autopsy case after death from extensive burn. The section of skin in which the bodies were most frequent is taken from the margin of the burn. The skin contains streptococci (*strept. pyogenes*), and small bacilli (*B. vulgaris*). This one control would throw out the majority of Mallory forms as being protozoa, unless two things were true; first, the child may have had scarlet fever; but this can practically be ruled out because no definite history of scarlet fever was obtained and no cases of infection followed. Second, the technic may not have brought out definitely what really existed, that is, the reticular bodies from the burn might show quite a different structure from those found in scarlet fever, with a different or yet undiscovered technic. In this case there were many transition stages from undoubted cytoplasmic degenerations to the definite reticular bodies, which is a strong point in favor of the cytoplasmic nature of all the bodies.

Among the control cases we examined pieces of skin from six living measles patients. In sections from these cases we have found focal areas of leucocytic infiltration of the intercellular spaces of the epithelium similar to those seen in the scarlet fever skins and occasionally we have seen a few small indefinite reticular bodies in the lymph spaces and in the epithelial cells, much more indefinite than most of those found in scarlet fever. In one case a very definite small reticular body with a central granule was seen in an epithelial cell, the reticulum staining a clear blue, the central granule a little deeper blue, with eosin and methylene blue staining; but of course little or nothing can be inferred from one form.

More characteristic of these measles skins is the presence of certain *hyaline* amoeboid forms found in the lymph spaces of the corium, especially beneath the areas of leucocytic infiltration of the epithelium. These forms have been seen in all the measles skins, but not in large numbers; they vary in size from that of a large coccus to that of a red blood cell. We have been unable to detect any definite structure in them, therefore they do not correspond with the small nucleated forms

seen by Field in measles. The forms described by Field we have not seen in the skin from the six living cases studied by us.

Besides these irregular hyaline bodies we have seen on the margins of the areas of leucocytic infiltration of the epithelium, lying between the epithelial cells, small collections of tiny delicate granules arranged in irregular groups and short rows. They are not definitely enough grouped or distinctly enough stained to be considered organisms, but they are in sufficient numbers to bear closer study. The measles cases have not yet been studied minutely enough to warrant a separate report. As controls for the scarlet fever skins, we can say that none of them show characteristic Mallory bodies.

In all of the sections of skin from the scarlet fever cases examined by us the reticular bodies found were in small definite areas. So it is possible that we may have missed some foci, but since many serial sections were examined in most of the cases we can say at least that the foci, if present, must be widely scattered. It is interesting to note that in one autopsy case a piece of skin taken immediately after death shows as many bodies as one taken twenty-four hours later. By referring to the table it will be seen that we found no relationship between the number of bodies and the presence of streptococci in the blood.

So far, then, in our study of these bodies, we have found no definite evidence of their being organisms; neither have we been able to show that they are merely degenerative tissue changes. It seems to us, however, that the evidence is in favor of this latter view of their nature.

Furthermore, we have been unable to demonstrate anything like a tiny organism within the larger bodies, therefore, we cannot agree with Prowazek in including scarlet fever in his group of diseases produced by what he calls Chlamydozoa. Prowazek's studies were made principally on vaccinia and trachoma. He has reported no studies on scarlet fever or rabies, and yet he includes both these diseases in his group. It seems to us that he has generalized from too few particulars.

No spirochetal forms were demonstrated by the Levaditi method.

Most of our work, up to the present time, has been on scarlet fever skins, but we feel now that more promising results may be obtained from a minute histologic examination of the exudates and of the superficial

tissues of mouth and nose, and possibly of the lymphatic system in general. We are, therefore, planning work along these lines for the immediate future.

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SOME CHARACTERISTICS OF THE STREPTOCOCCI FOUND IN SCARLET FEVER.*

BERTHA VAN H. ANTHONY, M. D.

The following work was carried on to determine the frequency of the occurrence of the hemolysing streptococcus in the blood, pus, blister fluid and throats of scarlet fever patients, its relative proportion to other streptococci present and the persistence of its hemolysing power. This study comprises a portion of the preliminary work upon the etiology of scarlet fever which is under investigation at the laboratory. By the term "hemolysing streptococcus" is meant that spherical or spheroidal organism which, by dividing in only one plane, grows in long or short chains, the individuals usually occurring in pairs, and which gives a more or less definite area of hemolysis about each colony when plated out in blood agar poured plates.

In 1903 Schottmüller, and soon after, Rosenow, showed that pneumococcus colonies gave green color in blood agar poured plates, streptococcus pyogenes gave hemolysing colonies and other streptococci (*streptococcus viridans*), green colonies. Reudiger (1906),† working with various throat cultures, found that pneumococci showed green and sometimes hemolysing colonies; streptococcus pyogenes, hemolysing colonies; other streptococci, green colonies. He also found that hemolysing colonies may show green color in subsequent plates, and that slightly hemolysing colonies and those with greenish cast, while very puzzling, should be distinguished from streptococcus pyogenes. Moreover, he found but a small proportion of the green colonies occurring in scarlet and other throat cultures to be pneumococcus. The others seemed to belong to a large group lying between streptococcus pyogenes and the pneumococcus, some being very like the streptococcus viridans of Schottmüller and others related to pneumococci.

Reudiger explains the green coloration in blood plates as due to the formation of lactic acid, which then acts on the sugars in the blood medium. In glucose-agar blood plates streptococcus pyogenes does

* This work was carried out under the direction of Drs. W. H. Park and A. W. Williams.

† Journal Infectious Diseases, 1906, Vol. III., p. 755.

give green colonies entirely, but none in blood and plain agar because it does not ferment muscle sugar readily and the hemolysis outruns the green coloration. In our laboratory all the hemolysing strains, and also the green ones, when tested and re-tested in glucose agar blood poured plates gave green colonies.

The plate method was the chief means of isolating the streptococci in pure culture from the scarlet fever material. In the case of *blood* about 5 or 6 c.c. were drawn from the veins of the arms of living patients, and in autopsy cases from the heart, by means of a sterile syringe. One c.c. was added to a test-tube of melted agar, which had been cooled to 41 degrees C., and the whole poured quickly into a sterile petri dish. The rest of the blood drawn was inoculated into a bottle or flask containing about 75 c.c. of broth, either plain or calcium (containing a few lumps of broken marble). The plate and broth were incubated twenty-four hours at 37 degrees C. If colonies appeared in the blood poured plates they were fished to blood agar slants. The broth was examined microscopically by smears stained with Loeffler's methylene blue, and also plated out by streaking a loopful over the surface of a freshly poured agar plate on which had been placed a loopful of sterile horse blood. The isolated colonies thus obtained were easily fished under the microscope to blood agar slants and tested shortly in blood poured plates, as follows:

From the agar slant a serum broth culture was made and after twenty-four hours incubation at 37 degrees C., dilutions were made by putting 0.25 c.c. in a second broth tube and of this mixture 0.25 c.c. in a third broth tube, and so on up to four or five dilutions. Then 0.1 c.c. from each of the latter dilutions was used to inoculate test tubes, each of which contained 1 c.c. of sterile horse blood. The plates were poured by adding to an inoculated blood tube a tube of melted agar already cooled to 41 degrees C. This gives a better mixture than pouring the blood into the melted agar as is necessary when a syringe is used as above.

Blister Fluid was obtained by applying strong ammonia to a small area of the skin where the rash was brightest, usually on the chest, by means of a disk of blotting paper surrounded by a circle of vaseline and held firmly in place by crossed adhesive strips arranged in a star form,

so that the air was practically excluded. After 10-15 minutes the fluid in the blister thus formed was drawn with sterile capillary pipets which penetrated the delicate epidermis easily. Cultures were made in serum broth and, after twenty-four hours incubation, treated as the blood cultures above.

The *pus* was drawn by a sterile syringe from suppurating glands or other foci of infection. Dilutions were made as above, but were plated out at once because the number of organisms present was usually much greater than in the blood.

Throat cultures were taken by means of sterile cotton swabs rubbed thoroughly on both tonsils. The swab of each case was beaten in a tube of plain broth and dilutions made as described before. At first ordinary broth tubes containing varying amounts of fluid were used and these incubated twenty-four hours before the blood poured plates were made. Later, tubes containing exactly 5 c.c. of broth were found more useful and 0.1 c.c. transferred from one to another. The dilutions thus made were plated out at once. It was found, by examining smears of the incubated tubes, that in the higher dilutions the streptococci showed a tendency to outlast other bacteria, so that in about the seventh or ninth dilution one had frequently a pure culture of streptococcus.

The following table gives in condensed form the number of cases examined and strains isolated in pure culture, together with the combined percentage of total number of streptococcus colonies present in the original material from the throats of early scarlet and measles cases.

Streptococci in Scarlet Fever.

Source.	Number of cases tested.	No growth.	Strept. isolated.	Blood Pour Plates.	
				Strept. Pyogenes (Hemolysing).	Other Strept.
Autopsy blood (from heart) ..	18	8	10	+	—
Living blood (from arm)	16	15	1	—	+
Total.....	34		11		
Autopsy pus.....	10	3	7	+	—
Living pus.....	9	0	9	+	—
Total	19		16		
Blister Fluid.....	26	23	3	+	+
Throats (scarlet)	35 (5 rejected.)	0	30	+ 27.3%	+ 72.7%
Controls: Measles Throats..	24	..	24	14.4%	85.6%
Diphtheria Throats.....	4	..	4	Fraction of 1%	+

* The calculation of a *percentage* of hemolysing colonies was begun only in the last half of the scarlet throats, but was carried out in all measles throats tested and varied considerably as follows:

Percentage of Hemolysing Colonies in Individual Cases.

Scarlet Throats.		Measles Throats.	
15 Cases.	Percentage.	24 Cases.	Percentage.
Scarlet fever 79.....	100.0	Measles 13.....	10.0
“ 80.....	0.0	“ 14.....	50.0
“ 81.....	0.2	“ 15.....	4.0
“ 82.....	0.7	“ 16.....	2.0
“ 83.....	10.0	“ 18.....	33½
“ 84.....	7.5	“ 19.....	2.0
“ 85.....	33½	“ 20.....	0.0
“ 86.....	0.0	“ 21.....	25.0
“ 87.....	0.0	“ 22.....	0.0
“ 88.....	80.0	“ 23.....	14.0
“ 89.....	17.0	“ 24.....	1.0
“ 90.....	70.0	“ 25.....	0.0
“ 91.....	91.0	“ 26.....	0.0
“ 96	40.0	“ 27.....	3.+
“ 97.....	50.0	“ 28.....	87.0
		“ 29.....	14.0
		“ 30.....	2.0
		“ 31.....	16.0
		“ 32.....	20.0
		“ 33.....	2.2
		“ 34.....	60.0
		“ 35.....	0.6
		“ 36.....	0.0
		“ 37.....	0.3

When the throat cultures of scarlet fever, measles and diphtheria were isolated by means of blood poured plates, the streptococci found showed considerable variation in reaction and the following terms were used in an attempt to classify the colonies:

1. Hemolysing, represented in charts by "A."
2. Small Hemolysing, represented in charts by "a."
3. Very Small Hemolysing, represented in charts by "a₁."
4. Slightly Hemolysing (large and small), represented in charts by "b."
5. Doubtful, represented in charts by "c."
6. Green (light and dark), represented in charts by "d."

Colonies were fished and replated during successive generations and it was found they varied up and down the scale:

Hemolysing colonies varied in area of hemolysis.

Green colonies varied in shade of green.

Small hemolysing colonies became *large* or *slightly hemolysing*, or *green*; this green in subsequent generations again became hemolysing.

Slightly hemolysing colonies remained the *same*, became *doubtful* or showed *green* in later plates.

Doubtful colonies became *slightly hemolysing*, remained *doubtful* or showed *green*.

Thirty streptococcus strains isolated from blood, pus, and blister fluid of scarlet fever cases were studied.

Autopsy Cases.		Living Cases.	
Case No.		Case No.	
13.....	{ Blood. Pus.	211.....	Blister.
50.....	Blood.	226.....	Blister.
53.....	{ Blood. Pus.	Sc. 22.....	Blister.
96.....	{ Blood. Pus.	51.....	{ Blood. Throat
204.....	Pus.	Sc. 93.....	Pus. }
206.....	Blood.	Sc. 95.....	Pus. }
212.....	Pus.	Sc. 98.....	Pus. }
Sc. 23.....	Blood.	Sc. 99.....	Pus. }
Sc. 24.....	{ Blood. Pus.	Measles and scarlet fever 102.	Pus. } Cervical glands.
Sc. 30.....	Blood.	Sc. 103.....	Pus. }
Sc. 48.....	Blood.	Sc. 104.....	Pus. }
Sc. 92.....	Pus.	Sc. 105.....	Pus. }
		Sc. 106.....	Pus. }

Controls: { No. C. 100 Bellevue Laboratory Culture.
 { No. C. 101 Cellulitis after erysipelas.

With one exception every *blood* and *pus* case in pure culture when tested in plain agar blood poured plates gave good hemolysing colonies. The *exception was the only strain isolated from living blood, and it gave green colonies.* The throat culture from this case also showed green colonies only. The patient from whom these were derived had a good rash, ran a typical course of scarlet fever and recovered *without complications.*

The three *blister fluids* which yielded streptococci showed both hemolysing and green colonies.

Re-test of Hemolysing Power of Above Strains.

The strains enumerated above (with the exception of Sc. 102, 103, 104, 105 and 106, which have only recently been isolated), and the two controls, making 27 strains in all, were kept in the ice-box on blood agar slants (being transferred at about four-week intervals), for three months to a year. They were then retested in successive blood poured plates and *showed marked variation as to hemolysis,* though all, except 51 Blood and Throat (which were green when isolated and remained so), had

been fished from colonies which showed good hemolysis in the original. The re-test was carried on as follows:

From a 24-hour blood agar slant of each strain a tiny portion of the growth was removed with a straight platinum needle and put in a 5 c.c. broth tube. This was shaken thoroughly and the two loopfuls of the broth transferred at once to a tube containing 1 c.c. of sterile horse blood. Melted agar was added and the plated poured as before. These plates were incubated eighteen to twenty-four hours and the colonies showing least amount of hemolysis were fished on blood agar slants which were plated out in turn. A careful record was kept as to the size and reaction of the colonies of each strain, only the most variable fishings being carried on from one plating to another. Each broth tube, from which an agar plate was made, was incubated and a smear from it examined, so no contamination could creep in unawares.

From the *hemolysing* colonies arose:

1. Hemolysing colonies of similar and smaller size.
2. Slightly hemolysing (large and small).
3. Doubtful colonies.
4. Green (light and dark).

In subsequent platings, as in the case of fishings from the throat cultures, there was a marked variation up and down the scale (see charts).

Hemolysing colonies varied in hemolysis, became slightly hemolysing, doubtful, green or showed no green nor hemolysis.

Slightly hemolysing colonies became hemolysing, remained slightly hemolysing or were doubtful, green or showed no green or hemolysis.

Green colonies remained green for the most part, but a few (see charts), returned to hemolysing, slightly hemolysing or were doubtful.

In two strains, 53 *Blood* and Sc. 48 *Blood*, at the fourth and third platings, respectively, there occurred among other colonies some which showed *neither hemolysis nor green color*. These were carefully fished and found in smears of broth transfers to be pure streptococcus. When these fishings were replated and refished one or more times they gave rise to *hemolysing, slightly hemolysing, green and doubtful colonies*, sometimes all in the same plate. Only a few remained the same, that is, showed no green nor hemolysis.

In the accompanying charts of *53 Blood and 53 Pus and *Sc. 48 Blood the variation is expressed as follows:

A+ = Large hemolysing—4-5 m.m. in diameter.

A = Medium hemolysing—3 m.m.

A₁ = Less hemolysing—2-2-1/2 m.m.

a = Small hemolysing—1-1/2 m.m.

a₁ = Very small hemolysing—1 m.m.

b+ = Slightly hemolysing (large).

b = Slightly hemolysing.

c = Doubtful.

d₁ = Light green.

d = Green.

d+ = Dark green.

E = No green, no hemolysis.

The figures above the letter (in the charts) indicate the succession of platings. These were usually at intervals of two or three days.

Eight fishings from colonies (of 53 Blood), which showed no green and no hemolysis were tested simultaneously in blood pour plates of plain agar, peptone water agar, and sugar-free agar (made from broth with colon bacillus added and re-sterilized), with the result that in the plain and peptone agar the hemolysis returned for the most part, while in the sugar-free agar the colonies remained the same or showed but slight trace of hemolysis.

* The charts of *Sc. 48 Blood* and *53 Blood* were so complicated that for the sake of clearness only a part of each chart is reproduced; the parts are typical, however, of the many fishings which were carried on from these same strains. The *53 Pus* chart is a simple one and is given entire. It is typical also of the other cases tested.

Blood Pour Plates.

Fishings of 53 Blood.	Plain Agar.	Peptone Agar Watery 1%.	Sugar-free Agar.
(1) E.....	E + b 60%	A (+ b)	E (+ b) 80%
(2) E.....	A	A + 2	E (+ b) 90%
3) E.....	A	A (+ b) 96%	E (+ b) 99%
(4) E.....	E (+ c) 95%	A (+ b) 90%	E
(5) E.....	A (+ b) few	A (+ b) 90%	E (+ b) few
6) E.....	A—(+ b) few	A—(+ a)	E
7) E.....	A (+ b) 80%	A (+ b) few	E (+ b) few
8) E.....	a	a	E
* Control.			
204 Pus d.....	d—	d—	d—

* The control in this test was a fishing (from 204 pus) which had remained green through five platings, and showed green colonies in all three media.

These "E" colonies (showing no green or hemolysis) were tested also upon:

Glucose agar which gave *green* colonies.

Glycerine agar which gave hemolysing and slightly hemolysing colonies.

Beerwort agar which gave deep green colonies.

Two strains, 13 Blood and Pus, were kept on glucose agar blood slants for a number of transfers at several day intervals and when changed suddenly to plain agar blood pour plates showed no loss of hemolysing power.

The two control cultures, one C100 from the laboratory of Bellevue Hospital and the other C101 isolated recently from a case of cellulitis following erysipelas, showed but little variation, for only two slightly hemolysing colonies occurred in the six platings of C100 and none in C101.

A re-test was made also of 20 fishings from *scarlet throats* as follows:

Hemolysing.	Slightly Hemolysing.	Green.
Scarlet fever, 24.		
Scarlet fever, 79.		
Scarlet fever, 81.....	Scarlet fever, 81.	
Scarlet fever, 82.....	Scarlet fever, 82.
Scarlet fever, 83.		
Scarlet fever, 84.		
Scarlet fever, 85 (small).		
Scarlet fever, 88.....	Scarlet fever, 88 (large).....	Scarlet fever, 87. Scarlet fever, 88. Scarlet fever, 89.
Scarlet fever, 90.		
Scarlet fever, 91 (small).		
Scarlet fever, 96.		
Scarlet fever, 97.		
Scarlet fever, 211.		
Scarlet fever, 212.		

Control Sc. 40 = from erysipelas case which developed rash and was suspected of being scarlet fever, but later proved negative.

These strains had been kept in the ice-box on blood agar slants for 12-18 months, and comprised not only hemolysing, but hemolysing and green colonies when first isolated.

The reactions of the originally *hemolysing colonies* followed practically those showed of the blood and pus hemolysing colonies when they were re-tested.

The colonies which had been *green* when isolated, remained so in every re-plating except once in the case *Sc. 87*, where doubtful colonies occurred in the third plate, but when these were fished and plated again they showed green and remained so for subsequent platings.

In the replating of the two *slightly hemolysing strains*, *Sc. 81* and *Sc. 88*, greater variation occurred as may be seen by the accompanying charts which show part of the fishings carried on in each case.

88 slightly hemolysing (large), gave: hemolysing, slightly hemolysing and a few doubtful colonies which, however, became hemolysing again. On the other hand *81 slightly hemolysing* gave but a low per-

centage of hemolysing colonies and these were small in size. The majority of colonies were slightly hemolysing with a few doubtful and a number of green ones. These last, the green, became small and tiny hemolysing colonies four times, but for the most part gave rise to slightly hemolysing and still other green colonies (see chart).

The *control* from the erysipelas throat which was hemolysing when isolated, gave in the re-test, *large* hemolysing colonies.

Although the power of hemolysing streptococci to cause hemolysis seems to be a slightly variable quality and is certainly dependent upon conditions we do not understand, yet there is a distinct tendency of this power to continue, even though the strains be kept on artificial media, for in the re-test the majority of colonies were hemolysing, the variation from the original strain being usually about 5 per cent., or less, in the first re-plating. In these and subsequent plates only the most variable colonies were fished and even then the percentage of hemolysing colonies was usually greater than 25 per cent., sometimes running between 75 and 100 per cent. All strains were plated out at least six successive times and some, eight or nine times. Any variation occurring cannot be due entirely to long cultivation on artificial media, partly because some recent green colonies derived from old hemolysing cultures kept on agar for seven to twelve months have, as is shown in the chart Sc. 48 Blood, regained their hemolysis once more. To be sure, when some of these apparently accidental green colonies, which formed but a very small part of all the colonies present, were selected strains were brought out which had lost their hemolysis completely. This occurred in $33\frac{1}{3}$ per cent. of the 27 blood and pus cultures retested, and these green strains failed to regain any appearance of hemolysis, though they were plated out six successive times (see chart of 53 Blood and Pus). It is doubtful if these can longer be classed with *streptococcus pyogenes*. On the other hand, as cited above in the chart of Sc. 48 Blood, some green colonies show a quick change to hemolysis again.

The occurrence of hemolysing streptococci (*strep. pyogenes*), as seen in the table, was almost constant in the material taken from scarlet, measles and diphtheria throats, while the *streptococcus viridans* was always present. In scarlet cases the hemolysing *streptococcus* alone occurred in the pus from cervical glands and other suppurating foci in

all of the nine living cases, and was recovered in seven of the ten autopsies. In the living blood and blister fluid streptococci would seem to be rather infrequent since they were found in but three out of twenty-six blisters and once in sixteen samples of living blood. It is interesting to note that the three children from whom streptococci, both hemolysing and viridans were isolated by blistering and also the man whose blood contained the streptococcus viridians, all recovered. Streptococci were not found in all cases of autopsy blood examined, being present in but ten of the eighteen tested and were of the hemolysing type.

53 Blood.

Typical Portion of chart for 53 Blood shortly after death, showing variation in hemolysing power of colonies in successive cultures from a markedly hemolysing streptococcus derived from heart's blood of a scarlet fever case after death.

- A+ = large hemolysing.
- A = medium hemolysing.
- A₁ = less hemolysing.
- a = small hemolysing.
- a₁ = very small hemolysing.
- b+ = slightly hemolysing (large).
- b₁ = slightly hemolysing (small).
- c = doubtful.
- d₁ = green (light).
- d = green.
- d+ = dark green.
- E = no green, no hemolysis.

53 Pus.

Whole chart, same case as 53 Blood.

Sc. 48 Blood.

Typical portion of chart showing variation in hemolysing power of colonies in successive cultures from a markedly hemolyzing streptococcus colony derived from

81 Throat. Typical portion.

81 Throat.

Duplicate chart, showing *percentage of variation* in hemolysing power of colonies in successive cultures from a *slightly* hemolysing streptococcus colony from throat of scarlet fever cases.

0 1 2 3 4 5 6

88 Throat.

Typical portion of chart showing variation in hemolyzing power of colonies in successive cultures from a slightly hemolysing streptococcus colony from throat of scarlet fever case.

ROUTINE DIAGNOSIS OF RABIES IN THE LABORATORY
OF THE NEW YORK CITY HEALTH DEPARTMENT
DURING THE YEARS 1906 AND 1907.

By ANNA W. WILLIAMS, M. D.

In the Research Laboratory, for the past two years, or since we have used the smear method in the routine diagnosis of rabies, there have been over 700 cases in all examined, including suspected rabies and controls.

These are divided into two groups, the first comprising the cases sent in for diagnosis, and the second the experimental cases.

When we began the work, in order thoroughly to test the smear method in diagnosis we used comparative tests on a long series of cases, including many diseases other than rabies. The comparative tests were animal inoculations and a study of sections. The results obtained from a study of 141 cases were published in January and May, 1906,* and our conclusions were that the Negri bodies as shown by smears, as well as by sections, are specific for hydrophobia, and that the smear method for examining the Negri bodies is more rapid and efficient than any other method published. Since then, in our routine work we have considered the presence of the Negri bodies in smears as diagnostic of rabies and have made no further control tests, except in those cases which we have used in our experimental work. Through this experimental work, however, we have added 119 cases to the list of those which had the comparative tests and our former conclusions have been more firmly established.

The following table gives the number of suspected animals sent for diagnosis each month during the years 1906 and 1907. Since, generally, only those animals that have bitten people are sent in for diagnosis, many cases of dumb rabies and of rabies with atypical symptoms are not diagnosed. Therefore, we can judge only roughly from these statistics as to the prevalence and increase of rabies in New York and vicinity.

* See in Collected Studies from the Research Laboratory, Vol. II., 1906.

TABLE I.

Animals Sent to Research Laboratory for Diagnosis.

Month.	1906.		1907.	
	Positive.	Negative.	Positive.	Negative.
January	7	1	18	3
February	5	1	12	6
March	9	1	12	2
April	5	1	9	5
May	12	1	13	3
June	10	4	11	7
July	3	7	10	5
August	9	5	19	6
September	7	2	21	8
October	9	8	14	5
November	11	1	24	4
December	14	5	18	5
Total	101	37	181	59

According to this table there has been a decided increase in the number of cases during 1907, but in interpreting these figures we must bear in mind that people are realizing more and more, especially since several prominent citizens have died from typical rabies, that such a disease really exists, and therefore, they are more particular about sending in material from suspicious cases. In the following table the animals received for diagnosis during 1907 are arranged according to the species and to the number of cases within and without the city.

TABLE II.

Kinds of Animals Received for Diagnosis During 1907.

Kind.	Locality Whence Received.			Diagnosis.	
	In City.	Out of City.	Total.	Positive.	Negative.
Dogs.....	187	31	218	168	50
Cats.....	7	2	9	4	5
Human beings..	5	1	6	6	..
Horses	4	..	4	2	2
Cows.....	..	2	2	..	2
Pigs.....	..	1	1	1	..
Total.....	203	37	240	181	59

Before analyzing the 573 cases examined since May, 1906, it may be well to state that by "Negri bodies" we do not mean the indefinite cell inclusions such as those found by Van Gieson in human meningitis, or by a few others in cats' brains, or by ourselves in dog and cat distemper. The more such indefinite bodies are found in other diseases, the greater is the evidence in favor of the specificity of the true Negri bodies.

Of the 537 cases, 335 are street cases and 238 experimental. Of the 335 street cases, 241 show typical Negri bodies and 94 show no definite forms. All of the latter, classed as doubtful cases, and 36 of the former were inoculated into animals. The 36 all gave positive results.

Of the 94 classed under doubtful cases, 12 brains were too decomposed to show anything; 4 of these were positive on animal inoculations; 10 were soft and showed suspicious bodies; 3 of these were positive; 7 were in good condition and showed suspicious bodies, 4 of these were positive, the 65 remaining ones showed absolutely no suspicious bodies and all were negative to animal inoculations.

We see, then, that in all of our work controlled by careful animal inoculations we have never yet failed to have typical rabies develop in animals inoculated with material showing definitely-structured Negri bodies.

Negative results after inoculation with such material must be interpreted by us at present as due to some error in technic, such as re-

gurgitation or hemorrhage at the time of inoculation, emulsion improperly made, not enough of the virulent material taken because of localization of the organisms, etc. Possibly individual resistance of the animal inoculated might play a part. We have used principally guinea pigs, and some of them have shown enough irregularity in regard to the time in which they have come down with the disease to suggest a varied individual susceptibility, if other factors can be ruled out.* So far, however, as I have stated, we have gotten no negative results with material showing Negri bodies.

No one has yet reported the finding of the characteristic structured bodies in any disease other than rabies. We give in the bibliography a list of works relating to the diagnosis of rabies which have appeared since May, 1906, as well as a few articles which were not included in our previous list (see J. Inf. Dis., 1906, Vol. III., 452). Lentz and Bohne have corroborated our methods of demonstrating the bodies. And all authors still agree that the typical Negri body is absolutely diagnostic of rabies.

On the other hand, some material in which we have failed to demonstrate typically-structured bodies has produced rabies. All of this material, however, since we improved our technic, has shown suspicious small forms similar to those found in rabbit fixed virus. But any decomposing brains may also show in smears, bodies very similar to these tiny forms, therefore, it is difficult to rule out rabies in such cases. Such cases have become less frequent with the improved technic, and if, as we believe, the Negri bodies are living organisms, there is strong reason to hope that in time we may clearly demonstrate in smears the presence of these tiny specific forms.

Until such a time, however, animal inoculations must be used in these cases. Of course, the animal test will probably always have to be used with brains that are too decomposed for the recognition of any formed elements except bacteria, unless a reliable chemical test can be discovered.

So far with us, *fresh* brains showing no Negri bodies and no suspicious forms have not produced rabies, but a few observers have

* The inoculations have all been made by the same operator, Mr. T. Deaken, and this may be the chief reason why our results with guinea pigs have been so uniform.

claimed that such material has produced the disease. Therefore, until we can reach a unanimous conclusion in regard to this point, we must in all such cases use animal inoculations. We may, however, be reasonably certain that a case showing such negative material was not a case of rabies.

It is possible that some of the animals from which this doubtful material was derived, killed early in the disease, before there had been time for general development of the virus in the brain, may have had virulent sputum. The virulence of the sputum is said to be due to the passage of the virus through the salivary glands. We are now carrying on a series of experiments to test the comparative virulence of these glands and the brain in true and doubtful cases. Our results are yet too few to be tabulated. We have, however, already had an interesting case in which this method was apparently helpful. A Russian wolfhound with typical symptoms of dumb rabies was sent in for diagnosis. The brain showed many small fixed-virus-like forms, which were considered suspicious. Guinea pigs were inoculated from brains and glands. Treatment was begun in a small child whose scratched hand had come in contact with the saliva. After twelve days, as no symptoms were observed in the guinea pigs, the treatment was discontinued. The pigs from the brain came down six and one-half weeks after the inoculation, while those from the glands are still alive ten weeks later. It was decided not to give more treatment to the child for this reason.

The question as to whether the *sputum* may be virulent and the salivary glands not, is an interesting one. It has been stated that the secretions from the mucous membranes of the mouth and naso-pharynx may also be virulent. If this were so, it would of course throw out negative results from gland inoculations. This question is now being investigated.

The localization of the Negri bodies is another important point in making diagnoses. We have found well-developed bodies distinctly localized in different parts of the brain in several instances. In one horse there were small, widely-scattered areas of well-structured forms throughout the cerebellum, while tiny, indefinite forms were scattered through the rest of the brain examined. In two human brains well de-

veloped forms were found in the corpus striatum and not in the rest of the brain. In several dogs the localization has also been marked.

The full technic of our present method of examination is as follows:

(1) The whole brain, and when wished, the Gasserian ganglion, spinal cord and its ganglia are removed under sterile precautions.

(2) The salivary glands are removed under sterile precautions.

(3) Small sections are cut from the various parts desired, especially from ammon's horn, cerebellum, and motor area of cortex. The sections are fixed in Zenker's fluid and embedded in paraffin, according to the method given in previous article.*

(4) Smears are made by pressing between a glass slide and a cover glass a small, thin section of the gray matter from (a) the cerebral cortex, (b) ammon's horn, or (c) the cerebellum; the material is spread along the slide by moving the cover glass down with the finger.

When partly or completely air-dried, the smears are fixed for about ten seconds in neutralized† methyl alcohol to which one-tenth per cent. of picric acid has been added. The excess of the fixative is removed by blotting with fine filter paper.

The fixed smears are stained with the following solution:

Saturated alcoholic solution of fuchsin..... 0.3 c.c.

Saturated alcoholic solution of methylene blue.. 2.0 c.c.

Distilled water 30.0 c.c.

NOTE—This solution changes rather quickly at room temperature, but kept in the icebox it gives good results for an indefinite time.

The stain is poured on the smear and held over the flame until it steams. The smear is then washed in tap water and blotted with fine filter paper.

With this stain the Negri bodies appear a magenta, the nerve cells blue, and the red blood cells yellow or salmon color.

(5) If nothing is found in the smears from the parts mentioned, smears are made from various other parts of the brain. If still nothing is found, an emulsion is made of good-sized pieces from the different

* Journ. of Inf. Dis., May, 1906.

† The wood alcohol is neutralized by adding sodium carbonate (Na_2CO_3) about .25 gms. to 500 c.c. of the alcohol.

‡ This mixture is practically the same as that recommended by Van Gieson for staining the Negri bodies.

parts of the brain and intracerebral inoculations are made into three guinea pigs, about one-fourth c.c. of the emulsion is inoculated. An emulsion is also made of the different parts of the brain in glycerine for later inoculations, if for any reason the first should fail. Sterilized, neutral glycerine is used. These emulsions remain active in the ice-box for over three months.

If one has a freezing ice-box, the whole brain[•] may be frozen, in which condition it will remain in a perfect state of preservation and fully virulent for over three months.

(6) Contaminated, doubtful material is made into a weaker fresh emulsion in order to lessen the number of bacteria. The glycerinated emulsion made from part of the same material is inoculated after two weeks, unless positive results have been gotten from the weak solution.

(7) One of the three guinea pigs is killed on the eighth day after inoculation, when, if the material inoculated was rabic the animal may show definite Negri bodies in the brain. Of course negative results are not definite. The other animals, if they do not develop rabies, are kept under observation for from four to six months.

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THE QUANTITATIVE CHANGES, DURING IMMUNIZATION,
IN THE BLOOD OF HORSES, AND THE RELATION
OF THE SERUMGLOBULIN TO DIPHTHERIA
AND TETANUS ANTITOXIN CONTENT.

DRS. EDWIN J. BANZHAF and ROBERT B. GIBSON.

Gravimetric determinations were recorded for the total and several individual proteins of sodium oxalate plasma fractionated with ammonium sulphate and sodium chloride. When precipitated the plasma-salt solution had a resulting volume of ten times the amount of the plasma employed. Coagulations were on aliquot portions of the filtrates, and the protein constituents (except serum albumin and in part the saturated NaCl soluble serum globulin), were calculated by difference. The eleven horses were subjected to an immunization, under Dr. Park's direction, against diphtheria and tetanus toxins simultaneously, each animal subsequently being continued on the toxin to which it responded best. Test bleedings, of about 500 c.c. only, were made until maximum antitoxic potency (with almost coincident greatest variation in content of the proteins), had been attained; afterwards routine bleedings were undertaken. The two refractory, one medium, and the eight horses producing a highly potent antitoxin all showed a maximum increase of from 40-114 per cent. for the serum globulin (for the refractory horses, 59.3 and 89.8 respectively). In one refractory and one highly antitoxic horse, the serum globulin maximum preceded the highest concentration in antitoxin; with three other horses, both maxima were attained when examined on the second bleeding. In seven of the horses, the greatest content in serum globulin was coincident with the maximum antitoxic potency. The greatest content in serum globulin was observed in the most potent plasma obtained in the series. The serumglobulin subsequently was maintained at a high concentration, roughly paralleling the antitoxic content in the plasma of the individual animals. The serumalbumin was diminished a half to a third along with the serumglobulin increase; though subsequent to the antitoxic and serumglobulin maxima, figures as low as a fifth the original albumin content were noted. The saturated NaCl soluble serum-

globulin was relatively increased from a normal 60-80 per cent. of the total serumglobulin to over 90 per cent.; in quantity, there is a rise of over 100 per cent., an increase of 163 per cent. being observed in one of the refractory horses. At dilutions of the plasma in the precipitated mixtures of 1:1.5, 1:5 and 1:10, the "euglobulin fraction" amounted to 60-70, 20-24 and 10-15 per cents. respectively of the total serumglobulin in both the normal and antitoxic plasma. In the 850 unit plasma, an increase in the "euglobulin" was noted, but the high content of proteins had probably influenced the precipitation limits. It would seem, then, that the "euglobulin" is not increased relatively to the total serumglobulin during the immunization as has at times been maintained. From the results we have obtained, it may be concluded that in forced immunization, the same characteristic quantitative changes can occur in the blood proteins, both of refractory horses and of those yielding a highly potent antitoxin.

THE FRACTIONAL PRECIPITATION OF ANTITOXIC SERUM.

By DRs. EDWIN J. BANZHAF and ROBERT BANKS GIBSON.

Comparatively little attention has been paid to the fractional precipitation of antitoxin. Brodie,¹ in 1897, separated antitoxin horse serum into four fractions by the progressive addition of ammonium sulphate of half saturation; all four contained, however, relatively equal amounts of antitoxin. Atkinson,² in this laboratory, saturated with sodium chloride a solution of the moist serum globulin precipitate obtained with magnesium sulphate, and by then employing heat differentiated the globulin into several fractions containing antitoxin. The protective properties corresponded roughly to the quantities of serum globulin in the precipitates. In some unpublished experiments he found that alterations of the amounts of coagulated proteid in the several fractions resulted if more magnesium sulphate was added before heating; there were proportionate changes in the distribution of the antitoxin. Owing to the destruction of a portion of the antitoxin at the higher temperature and possible injury by exposing it to heat of less degree, this fractionation must be considered as incomplete and does not exclude a purification of the antitoxin by salt fractionation. The work of E. P. Pick on the ammonium sulphate fractioning of the antibodies has been referred to in the preceding communication. Our own experiments have resulted somewhat differently from either those reported by Atkinson or by Pick, and have developed some new and suggestive facts.

On the basis of the solubility of the antitoxic proteids in saturated sodium chloride solution, one of us (Gibson), recently devised a method for the partial purification and concentration of antitoxin.¹ This consisted in precipitating the diluted plasma with an equal volume of saturated ammonium sulphate and separating the antitoxic proteids by extracting the precipitate with saturated sodium chloride solution. We

¹ Brodie: *Journ. of Path. and Bact.*, iv, p. 460, 1897.

² Atkinson: *Journ. of Exper. Med.*, v, p. 67, 1901.

now have employed the method of salt fractionation to study further the concentration of antitoxin.

There exists at the present time considerable confusion in comprehending the methods and basic principles of ammonium sulphate fractional precipitation of proteids. The nomenclature which we have employed and which designates the number of c.c. of saturated ammonium sulphate solution in 10 c.c. of the *precipitated* mixture has been used by some authorities; it avoids the confusion developed by the use of such terms as "per cent $(\text{NH}_4)_2\text{SO}_4$ solution" and "per cent saturation $(\text{NH}_4)_2\text{SO}_4$ " "per cent of saturated $(\text{NH}_4)_2\text{SO}_4$ solution" "per cent saturation $(\text{NH}_4)_2\text{SO}_4$ solution" and it seems the simplest and best *practical* expression of degrees of saturation yet suggested. We advise that this method be employed in future papers on fractional precipitation.

Mann, in his version of Cohnheim's "*Chemie der Eiweisskörper*," states (p. 292): "As the solubility of ammonium sulphate is 76.8° (per cent?) at room temperature, it is easy to calculate what percentage of ammonium sulphate is required for bringing about incipient and complete precipitation of any one albumin, as soon as we know what amounts of saturated ammonium sulphate have to be added for any given quantity of fluid." The simplicity of the above method of calculating vanishes when attention is drawn to the fact that while 100 parts of water dissolve 76.8 gms. of dry ammonium sulphate, the volume resulting is increased to 141 c.c. so that 100 c.c. of the saturated solution actually contain approximately 54 gms. of the salt, and the degree of saturation as indicated by the *content* of dry ammonium sulphate must be calculated with reference to the latter figure. An example will make clearer the above statement: To obtain a concentration of "half saturation" ammonium sulphate, equal volumes of the proteid solution and of saturated ammonium sulphate solution are mixed; according to the apparent meaning of Mann's obscure statement, 100 c.c. of the resulting "half saturated" solution would contain 38.4 gms. of the dry salt; it actually does contain 27 gms. of ammonium sulphate.²

E. P. Pick has fallen into the same error in his paper on the fractionation of the anti-substances in the globulins of serum. He speaks (p. 356) of the limits of the various serum fractions as follows: "dass das von Reye aus normalen Pferdeserum abgeschiedene Fibrinoglobulin entsprechend einer Sättigung von 21.5 Proz. Ammonsulfat.....ein bestimmter Teil (the euglobulin) des nun übrig bleibenden Globulins keine antitoxische Wirkung hatte und dass sich dieser aus dem Serum noch bequem abschieden liess, wenn die Flüssigkeit einen Gehalt von 25.6 Proz. an Ammonsulfat enthielt. Es verblieb nunmehr ein Eiweisskörper in Lösung (the pseudoglobulin) der durch weiteres Eintragen der gestättigten Ammon-sulfatlösung bis zu einem Gehalte von 38 Proz. von dem Serumalbumin gut zu trennen ist und den Heilkörper in quantitativer

¹ The literature on the purification and chemical characters of anti-bodies has been briefly reviewed in a paper on "The Practical Concentration of Diphtheria Antitoxin for Therapeutic Use," this *Journal*, i, p. 161, 1906, and more recently by Ledingham: *Journ. of Hyg.*, vii, p. 65, 1907.

² Because of the change in the volume of the solvent on adding the salt, it is similarly not possible to add 38.4 gms. of ammonium sulphate to 100 c.c. of water and have a solution at "half saturation." In this case the volume would be increased to 120.7 c.c. and 100 c.c. would contain 31.7 gms. of the salt.

Ausbeute enthält." The precipitation limits are distinctly designated here by 21.5, 26.6 and 38 per cents of ammonium sulphate in the precipitated mixture. They actually mean a content of 2.9, 3.33, and 4.9 c.c. of saturated ammonium sulphate solution in 10 c.c. of the precipitated mixtures, which would then contain, respectively, 15.67, 18.00 and 26.50 gms. of the dry ammonium sulphate per 100 c.c.—figures which by no means or method of interpretation can be logically expressed by 21.5, 25.6 and 38 percentages of ammonium sulphate. Fortunately the fault lies in the nomenclature only, the precipitations being accomplished by the use of saturated ammonium sulphate solution.

Twenty liters of plasma (475 units per c.c.), were diluted with 20 liters of water, by fractioning with saturated ammonium sulphate solution, the three proteid precipitates were obtained which separated at concentrations corresponding to 3.3 c.c., 3.3—3.8 c.c. and 3.8—5.0 c.c. of the saturated salt solution in 10 c.c. The saturated sodium chloride soluble antitoxic) globulins of these fractions and of the 5.0 saturation precipitate of a second 20 liters of the plasma were prepared as usual. Proteid determinations (coagulations) and potency tests were duplicated.

Prep. 77.

Fractions.	A. 0.0-5.0	B. 0.0-3.3	C. 3.3-3.8	D. 3.8-5.0
Volume c.c.....	5,200	1,440	1,400	2,050
Units per c.c.....	1,450	1,150	1,350	1,750
Times concentrated.....	3.05	2.42	2.84	3.68
Per cent recovered.....	79.3	17.4	19.9	37.8
Proteid, gms. per 100 c.c.....	11.66	11.51	9.87	9.70
Units, per gm. proteid.....	12,436	10,000	13,666	18,000

A second experiment with a 450 unit plasma gave the following results:

Prep. 82.

Fractions.	A. 0.0-5.0	B. 0.0-3.3	C. 3.3-3.8	D. 3.8-5.0
Volume c.c.....	6,240	1,350	1,640	2,550
Units per c.c.....	1,050	900	1,300	1,600
Times concentrated.....	2.34	2.00	2.89	3.56
Per cent recovered.....	72.8	13.9	22.6	45.3
Proteid, gms. per 100 c.c.....	10.59	12.06	13.46	13.41
Units, per gm. proteid.....	9,914	7,464	9,655	11,930

These observations show that the antitoxic globulins of the higher fraction are much more potent than those of the less soluble proteids.

Both the preparations by the half-saturation ammonium sulphate method and by fractioning, when precipitated from the saturated sodium chloride solution and dialyzed, contained a probably partially denaturalized antitoxic globulin; this had a diminished solubility and antitoxic potency (per gram proteid) and was precipitated on slight acidification by diluting twenty times. The filtrates from the water-acid precipitable globulin coagulated at 73 degrees, while saline solutions of the precipitates so obtained showed varying and much lower coagulating temperatures. The solutions of the high proteid fractions have a peculiar green color. A redetermination of the precipitation limits of the globulin in the three fractions after removal of the water-acid precipitable proteid, showed that the different precipitation limits were relatively characteristic for the fractions.

The following results were obtained on progressively fractioning (in two experiments) by the addition of the dry salt¹ to a liter of about 400 units antitoxic plasma. The initial dilution was 1:5. The precipitates were pressed between filters and extracted with saturated sodium chloride solution. The determinations on the filtered extracts are given per c.c. of the original plasma. The results are roughly quantitative only, loss of the filtrate in pressing out the precipitated globulins being disregarded. Proteid determinations and potency tests were duplicated.

¹ Calculations or reference tables for the amounts of salt to be added to produce or raise a proteid solution to any desired concentration may accurately be made by employing the following formula:

$$X = \frac{v p (c_2 - c_1)}{10 - e p c_2} \quad \text{where } x \text{ is the number of gms. of salt to}$$

be added to give the required concentration, v the original volume in c.c., e the increase in the volume of the solvent by 1 gm. of salt, p the gms. of salt per c.c. of its saturated solution, and c_1 and c_2 are the initial and desired degrees of saturation, expressed as c.c. in 10 c.c. For $(\text{NH}_4)_2\text{SO}_4$ e and p may be regarded as approximately 0.54; then

$$X = \frac{v (c_2 - c_1)}{18.158 - 0.54 c_2}; \quad \text{and when } c_1 = 0, \quad X = \frac{v c_2}{18.158 - 0.54 c_2}$$

To raise the concentration by the addition of saturated salt solution the amounts (c.c.) of the original proteid solution and of the saturated salt solution in the mixture are calculated; also the amount of the salt solution necessary to bring the *proteid solution* to the desired concentration. Sufficient excess of saturated salt solution over that already present is added to make the required total.

Fractioning of Plasma 305, 8/1/06.

Fractions.	Proteid per c.c.	Units per c.c.	Units per Gram of Proteid.
A			
0.0-3.4	0.00321	25	7,788
3.4-3.6	0.00223	20	8,968
3.6-3.8	0.00432	47	10,879
3.8-4.0	0.00416	52	12,500
4.0-4.2	0.00408	60	14,705
4.2-4.4	0.00272	55	20,220
4.4-4.6	0.00191	40	20,942
4.6-4.8	0.00163	32	19,632
4.8-5.0	0.00111	19	17,117
5.0-5.6	0.00428	18	4,205
B			
0.0-3.4	0.00394	27	6,853
3.4-3.6	0.00219	20	9,132
3.6-3.8	0.00397	45	11,335
3.8-4.0	0.00336	50	14,880
4.0-4.2	0.00332	60	18,072
4.2-4.4	0.00225	55	21,568
4.4-4.6	0.00181	40	22,094
4.6-4.8	0.00147	30	20,408
4.8-5.0	0.00093	18	19,355
5.0-5.6	18

In each instance there is a progressive increase in potency as the antitoxic globulin becomes more soluble in the fractions until a concentration of the salt of about 4.2 is reached. The potency per gram remains then practically constant at about three times that of the saturated sodium chloride extract of the euglobulin fraction (0.0—3.4) until between 4.8 and 5.0 saturation; above this limit the potency per gram rapidly diminishes to a relatively very low figure. Between the 4.2 and 4.8 limits, over half the units of the original plasma are precipitated, while the antitoxin is contained in less than one-third of the total antitoxic globulin.

The fact that the major portion of the antitoxin remained soluble at a concentration of 4.2 saturation, led us to investigate whether the

protective material was mechanically precipitated with the proteid of the lower fractions. Such a result seemed *a priori* improbable because the individual fractions were at such frequent intervals and contained such a small amount of the globulin precipitate as to make hardly conceivable a mechanical inclusion of more soluble colloidal particles for more than a few minutes' duration. Our plan was to fraction the antitoxic plasma at 4.2 saturation. The lower fraction (precipitate) was to be dissolved in an added known volume of water, reprecipitated at 4.2 saturation and after standing 24-48 hours was to be filtered. The procedure was twice repeated with the precipitates obtained at 4.2 saturation. The three filtrates and the saturated sodium chloride soluble (antitoxic) globulin of the final 4.2 saturation precipitate were examined for globulin and antitoxic content. We were not able to separate by three times repeated fractioning at 4.2, the antitoxin from the lower fraction; over half the antitoxin brought down at first was pronouncedly a constituent of the precipitate, the amount in the filtrate from the final precipitation being very slight (though the potency per gram of proteid was relatively high). The protocol follows:

250 c.c. of antitoxic plasma (305, 8/10/06, 300+units per c.c.) were diluted with 475 c.c. of water and precipitated with 525 c.c. of saturated ammonium sulphate solution. After standing 24 hours, the precipitated globulin was filtered off. To the filtrate, 1000 c.c., was added 60 gms. of dry ammonium sulphate after sufficient ammonium sulphate solution had been employed to give 1500 c.c. at half saturation. The resulting precipitate (4.2-5.5 saturation) was pressed out between filters, dissolved and made up to 200 c.c.

The precipitate at 4.2 saturation was pressed out between filter paper, dissolved by the addition of 580 c.c. of water and reprecipitated with 420 c.c. of saturated ammonium sulphate solution. The total volume of the precipitated mixture was slightly over 1000 c.c. After standing 24 hours, the reprecipitated globulin was filtered off from "Filtrate I" (900 c.c.).

The precipitate from I was dissolved in 580 c.c. of water and precipitated with 420 c.c. of saturated ammonium sulphate. Filtrate II was 930 c.c.

Filtrate III similarly obtained amounted to 950 c.c. The globulin precipitate was extracted with 1000 c.c. of saturated NaCl solution.

Determinations of proteid and antitoxic content are given per c.c. of the original plasma.

	Proteid per c.c.	Units per c.c.	Units per Gram of Proteid.
Filtrate I.....	0.00270	40	14,810
" II.....	0.00332	50	15,080
" III.....	0.00075	12	16,100
4.2 Ppt. gbl. (ext. NaCl).....	0.01066	125	12,100
4.2—5.6.....	0.00685	80	11,680
	0.02428	307	

Further fractioning after complete removal of the water precipitable globulin was done on 50 c.c. of the globulin solution, Prep. 77A (cf. p. 255). The fractioning was made at a dilution of the original preparation of 1:20. The results are expressed per c.c. of the original undiluted preparation.

Refractionation of Preparation 77a.

Fraction.	Proteid per c.c.	Units per c.c.	Units per Gram of Proteid.
0.0—4.0	0.0408	400	9,791
4.0—4.4	0.0165	225	13,667
4.4—5.0	0.0176	375	21,306
5.0+	0.0018	75	41,722
4.8—5.5*	0.0046	150	34,783

* Made on a second 50 c.c. of the same preparation.

The refractioning of 77A from which the water-acid precipitable globulin had been removed, showed a marked progressive increase in potency hand in hand with the greater solubility of the proteid.

Fraction 3.8—5.0 of Prep. 82 was refractioned without removing the water-acid precipitable globulin. The dilution was 1:10.

Refractionation of Preparation 82d. (High Potency Fraction.)

Fraction.	Proteid per c.c.	Units per c.c.	Units per Gram of Proteid.
0.0—4.0	0.07318	600	8,136
4.0—4.2	0.91779	240	13,490
4.2—4.4	0.02197	260	11,840
4.4—4.6	0.01232	160	12,990
4.6—4.8	0.00708	90	12,711
4.8—5.0	0.00511	80	15,670
5.0—5.6	0.00197	90	45,690
	0.13941	1,510	
For 82D.	0.1341	1,600	

82D contained a globulin of rather uniform potency per gram from fractions 4.0—4.8; then a marked jump for the fraction 5.0—5.6 to about three times the original potency per gram was observed. The portion of antitoxin in the highest fractions of 77A and 82D was less than 6 per cent. of the total units. Prepared for administration as is the ordinary antitoxic globulin, the resulting product would have had a potency of from 5000—6000 units per c.c.

The high antitoxic potency per gram proteid of the globulin in the preparations precipitable between 4.8 and 5.6 saturation led us to attempt to obtain such a product in bulk from the antitoxic globulin preparations (Gibson) and directly from plasma. Our experiences have comprised (1) the influence of the reaction of the plasma, (2) repeated extraction (with 4.8 saturation ammonium sulphate) of the globulins to dissolve out the mechanically precipitated highly potent antitoxic substances, and finally (3) progressive denaturalization of the globulin by repeated extractions with saturated sodium chloride solution and reprecipitation with the sulphate. Our results, however, have not been encouraging. The protocols are given below:

(1) a. 250 c.c. of antitoxin plasma (262, 10/22/6, 500 units per c.c.), were diluted with 1050 c.c. distilled water and precipitated at 4.8 with 1200 c.c. of saturated ammonium sulphate solution. After standing three hours it was filtered and the filtrate (2250 c.c.) raised to 5.0 saturation by adding 28.6 gms. of dry $(\text{NH}_4)_2\text{SO}_4$. After 24 hours at room temperature, the half saturation precipitate was filtered off, pressed between filters and made up to 225 c.c. Of the 5.0 saturation filtrate, 2200 c.c. were precipitated at 5.6 saturation with 96 gms.

of dry (NH₄)₂SO₄ and filtered after 24 hours' standing at room temperature. The precipitate was pressed out and made up to 218.5 c.c. in distilled water.

b 250 c.c. of the same plasma were made distinctly alkaline with $\frac{N}{10}$ NaOH and then fractioned exactly as in *a*.

c. 250 c.c. of the plasma were made distinctly acid with dilute acetic acid, and then similarly fractioned.

Proteid coagulations and potency tests were made as usual.

Plasma.	Fractions.	Proteid per c.c.	Units per c.c.	Units per Grams of Proteid.
<i>a</i> . Native.....	4.8-5.0	0.0015	11	7,333
	5.0-5.6	0.0051	20	3,921
<i>b</i> . Alkaline.....	4.8-5.0	0.0011	11	10,000
	5.0-5.6	0.0947	20	4,255
<i>c</i> . Acid.....	4.8-5.0	0.0012	11	9,175
	5.0-5.6	0.0060	24	4,000

(2) 500 c.c. of the plasma of the bleeding employed for reprecipitation at 4.2 (305, 8/10/06, 300 units per c.c.)¹ were diluted with 800 c.c. of water and precipitated at 4.8 saturation with 1200 c.c. of saturated ammonium sulphate solution. After standing for 24 hours, the precipitate was filtered off. Of the filtrate, 2250 c.c. were precipitated, at about 5.6 saturation, with 116 gms. of dry (NH₄)₂SO₄; after standing, the precipitate was separated, pressed out between filter paper and made up in solution to 450 c.c. with water (fraction 4.8-5.6). The moist precipitate obtained at 4.8 saturation was thoroughly suspended in about 1500 c.c. of 4.8 saturation (NH₄)₂SO₄ and filtered after standing for two days, during which time the mixture was occasionally shaken up. The precipitate from the first filtrate (I) was re-extracted as before, this procedure being carried on, in all, four times. The precipitate then remaining was made up with saturated NaCl solution to a volume of 1000 c.c. Proteid determinations and the antitoxin tests were made on the fraction 4.8-5.6, on the four filtrates and on the NaCl extract of the residue, and are tabulated as before per c.c. of the original plasma.

	Proteid per c.c.	Units per c.c.	Units per Gram of Proteid.
4.8-5.6 sat.....	0.00191	12*	6,316
Filtrate I.....	0.00240	10*	4,170
“ II.....	0.00160	9*	15,000
“ III.....	0.00026	8*	30,770
“ IV.....	0.00030	4*	13,333
Residue.....	0.01784	250	14,020
	0.02431	293	

* Because of the low antitoxic and high (toxic) (NH₄)₂SO₄ content, the tests were made with 25 or 50 m.l.d. instead of the 100 m.l.d. ordinarily employed.

¹ Cf. pp. 101 and 102.

The amount of antitoxin and of proteid in the filtrates was so small that slight errors in the determinations would influence greatly the calculations of the antitoxin units per gram of proteid. Yet the results obtained on Filtrate III., when the figures on the preparations are recalled (p. 259), make it highly probable that a very small portion of the antitoxin can be separated in a much more highly potent form than is the case for the bulk of this substance.

Progressive denaturalization of the proteid as a means of separating the globulin from the antitoxic substance, if other than the serum globulin itself, has not proved successful. The method used was to extract the ammonium sulphate globulin precipitates of plasma or the antitoxic globulin preparation with saturated sodium chloride solution, to filter off the insoluble globulin residue (after some days standing), and to reprecipitate the filtrate by the addition of a little over half its volume of saturated ammonium sulphate solution. The extraction of this last precipitate with the sodium chloride and the precipitation of the filtrate with ammonium sulphate followed. This procedure was carried on 4—6 times on the 4.8 residue of (2), and on two antitoxic globulin solutions, which were obtained by the sodium chloride extraction of 3.8 saturation precipitates (less potent fraction), one of which was already thoroughly denaturalized in preparation because of the accidental partial desiccation of the acidified saturated sodium chloride precipitated proteid before dialysis. The final filtrates of one of the sodium chloride extracts of the originally denaturalized antitoxic globulin preparations had a potency of almost 15,000 units per gram of proteid. The globulin solution of the 4.8 saturation extraction precipitate and the second antitoxic globulin preparation contained about 10,000 units per gram of proteid.

The injection of the antitoxic globulins of the various globulin preparations sensitizes guinea pigs to subsequent, otherwise non-fatal intraperitoneal administration of serum (Smith and Rosenau and Anderson). Injected intraperitoneally into sensitized guinea pigs, the typical convulsions produced by serum are incited, and the deaths of the animals may ensue. Rashes of the urticarial character with little or no accompanying constitutional symptoms may follow the therapeutic administration of the several fractionally precipitated antitoxic globulins.

Therapeutically there is no difference in the results obtained with the equivalent unit injections of either the high (3.8 + saturation) or low (3.3 saturation), fractions of preparations 77 and 82 (pp. 255 and 256).

Conclusion.

From the data presented, it appears that the *saturated sodium chloride soluble* serum globulins of the higher fractions are uniformly much more potent per gram of proteid in antitoxin than are those precipitated by lower concentrations of ammonium sulphate. Between concentrations of the sulphate of 5.0 and 5.6, a small proportion of the total sodium chloride soluble globulin of the antitoxic globulin preparation (Gibson) or of a higher fraction of the same is precipitated; the solution of this globulin has a protective power of over 40,000 units per gram of proteid. The direct fractioning of the plasma, however, does not yield so potent a product; at a dilution of 1:5 of a 400 unit plasma the globulin remaining in solution at 4.2 and precipitated at 4.8 saturation has a potency of about 20,000 units per grain of proteid. It is thus practicable to prepare an antitoxic solution of over 2,000 units per c.c. from a relatively low plasma.

Whether or not this difference in the potency per gram of proteid is associated with the presence of non-antitoxic globulins having the same fractional precipitation limits as the protective substance remains as yet undecided. It is possible that such a variation in potency may be purely physical, associated with the size or condition of aggregation of the colloidal globulin particles—the less soluble larger masses having diminished antitoxic properties. Certainly, however, we find the antitoxin is characterized by a wide range of the precipitation limits similar to the soluble globulins, *i. e.*, in spite of repeated precipitations, a part of the antitoxin is comparatively insoluble in concentrations of ammonium sulphate in which the major portion of the protective substance readily dissolves.

In concluding the present paper, we desire to express our appreciation of Dr. Park's suggestions and helpful criticism.

ON THE FRACTIONATION OF AGGLUTININS AND ANTI-TOXIN.

By DRS. ROBERT BANKS GIBSON and KATHARINE R. COLLINS.

E. P. Pick,¹ in 1901, associated a number of antistances individually with the one or the other of the two serum globulin fractions of the Hofmeister classification. In the pseudoglobulin (3.4 to 4.6 saturated ammonium sulphate solution)² group of antibodies he placed the diphtheria and tetanus antitoxins and the typhoid agglutinin of horse serum; the lower or euglobulin fraction (2.9 to 3.4 saturated), comprises diphtheria and tetanus antitoxin and cholera lysin in the goat, rabbit and guinea pig, and finally cholera agglutinin in the horse and goat. It becomes possible, according to Pick, to separate the individual specifically reacting antistances by fractioning appropriate mixtures of sera. Such a possibility suggested the application of this method to the further study of certain antibodies, especially of the relation of specific and group agglutinins developed by immunization against a single strain of organism. Preliminary experiments in the course of our investigation indicated the unreliability of Pick's differentiation, and attention was accordingly directed to the actual possibility and practicability of distinguishing between antibodies by fractionation of the globulin. The availability of polyagglutinative sera for the work gave a chance for making numerous and extended observations of the distribution of these antibodies in the fractions.³

¹ *Beitr. z. chem. Physiol. u. Path.*, i, p. 351, 1901.

² The degrees of saturation, as here expressed, indicate a concentration equivalent to a content in 10 c.c. of the precipitated solution of 3.4 and 4.6 c.c. of saturated ammonium solution respectively.

³ A preliminary account of our results was published several months ago in the *Proceedings of the Society for Experimental Biology and Medicine*, iv, p. 15, 1906-1907.

The literature on the fractional precipitation of the antibodies is not extensive. Ide and Lemaire¹ (in 1899), found the precipitation limits of diphtheria antitoxin in horse serum to be from 2.8—4.4 saturation. Fuld and Spiro² (1900), associated the antirennin of horse serum with the pseudoglobulin, while a milk-coagulating action was possessed by the eu-fraction. The failures of Brodie and of Atkinson (of this laboratory), to separate diphtheria antitoxin from the accompanying serum globulins are referred to in the following paper. Porges and Spiro,³ without giving any of their experimental protocols divide, according to the distribution of the antibodies, the serum globulin into three distinct fractions; the ammonium sulphate precipitation boundaries of these overlap unless the serum is greatly diluted. Landsteiner⁴ found that the antitryptic action of blood serum is possessed by the albumin precipitated by complete saturation with ammonium sulphate after removal of the globulin. Cathcart⁵ also observed the antitrypsin to be associated with the albumin but not with the globulin fraction. Glaessner⁶ states that the euglobulin fraction inhibits the action of trypsin, but the typical protocol which he publishes and his statement of the Hofmeister classification show a misconception and confusion of the identity of his fractions.⁷ Glaessner apparently found that the globulin remaining in solution on dialysis was antitryptic.

Very recently Simon, Lamar and Bispham⁸ found that the opsonic substance in blood serum was precipitated with the serum proteids

¹ Ide and Lemaire: *Arch. internat. d. pharmacodyn.*, vi, p. 477, 1899.

² Fuld and Spiro: *Zeitschr. f. physiol. Chem.*, xxxi, p. 133, 1900.

³ Porges and Spiro: *Beitr. z. chem. Physiol. u. Path.*, iii, p. 277, 1903.

⁴ Landsteiner: *Centralbl. f. Bakt.*, xxvii, Abt. I, p. 357, 1900.

⁵ Cathcart: *Journ. of Physiol.*, xxxi, p. 497, 1904.

⁶ Glaessner: *Beitr. z. chem. Physiol. u. Path.*, iv, p. 79, 1904.

⁷ The error on his part has not heretofore been noted. Glaessner states (p. 82): "Das Globulin des Blutserums lässt sich nach den in Hofmeisters Laboratorium in mindestens 3 Fractionen zerlegen: in das bei 25 Proz. Sättigung mit Ammonsulfat ausfallbare Fibrino-globulin, in das Euglobulin, das bei einer Sättigung von 33 Proz. ausfällt und bei der Dialyse in Lösung bleibt, und endlich in das bei 38 Proz. Sättigung ausfallbare bei der Dialyse unlösliche Pseudoglobulin." The confused nomenclature of the degrees of salt saturation is discussed in the preceding paper (p. 98).

⁸ Simon, Lamar and Bispham: *Journ. of Exp. Med.*, viii, p. 651, 1906.

which separated out on dialysis. Opie and Barker¹ observed that proteolysis in an alkaline medium by the enzyme of the leucocytes is inhibited by the serum albumin.

In a paper just published on the relation of diphtheria antitoxin to the serum globulin, Ledingham² finds that the pseudo-globulin of horse serum contains the greater part of the antitoxin. The repeatedly precipitated euglobulin, however, in one horse still contained fully 10 per cent. of the antitoxin; in a second horse, the euglobulin similarly treated contained practically none of the antitoxin. Single precipitations (without further purification) showed that large amounts of antitoxin may be carried down with the lower fraction; with the one horse, judging in part from the tests on the pseudoglobulin fraction, over half the units must have precipitated with the euglobulin.³ Ledingham also confirms our own observation here reported that the diphtheria antitoxin of the goat is not invariably linked to the euglobulin fraction as maintained by Pick.

Recently observations have been made by Ruediger⁴ on the relation to the blood proteids of streptolysin, the hemolytic substance produced by the development of streptococci in heated serum. The lysin was precipitated with the globulin by saturation with magnesium sulphate; it was found with both the euglobulin and pseudoglobulin of the fractionated undiluted serum and also in both the insoluble proteid and the filtrate of the dialyzed half saturation ammonium sulphate precipitate.

¹ Opie and Barker: *Journ. of Exp. Med.*, ix, p. 207, 1907.

² Ledingham: *Journ. of Hygiene*, vii, p. 65, 1907.

³ Brieger (*Festschrift für R. Koch*, Jena, 1903) and also one of us (Gibson) have already reported similar experiences. In some as yet unpublished experiments carried on for another purpose by one of us (Gibson) and E. J. Banzhaf of this laboratory, it has been found that if undiluted horse serum be precipitated with half its volume of saturated ammonium sulphate solution and allowed to stand for 18 hours, the euglobulin precipitate may contain over two-thirds of the total serum globulin. Precipitation under the same conditions except that the precipitated mixture is 5 or 10 times the volume of the original serum gives a euglobulin figure only of from a fifth to a third the total globulin. The euglobulin at a dilution of 1:10 is noticeably smaller than at 1:5. This fact explains the diminished antitoxic content of reprecipitated or washed euglobulin fraction and makes difficult any hard and fast division into eu- and pseudo-globulins.

⁴ Ruediger: *Journ. of Infect. Diseases*, iv, p. 377, 1907.

Moll,¹ however, has shown that such heating suffices to alter the chemical composition and to change the precipitation characters of the blood proteids.

Owing to the difficulty of making a hard and fast division of the serum globuline into the eu- and pseudo-fractions, our experiments were not planned to be interpreted especially from the quantitative occurrence of the agglutinins in the one or the other fraction of specific agglutinating sera. We have aimed, however, to determine if the relative proportion of the agglutinins of polyagglutinative sera in the fractions remained constant as regards the proportional distribution of all the agglutinins of the serum in the eu- and pseudo-globulins. In a word, by a difference of precipitation limits to ammonium sulphate, it should be expected that the bulk of one or more of the agglutinins would appear in the one fraction, as contrasted with the larger proportion of each of the remaining agglutinins occurring in the other fraction. Attention is particularly directed in studying the results from our standpoint to the pseudoglobulin fraction (filtrate from the euglobulin fraction). Any loss from the euglobulin fraction through solubility in the wash solution has been considered only in the case of the antitoxins. Such loss may be interpreted as due to the resolution of the mechanically precipitated proteids of the more soluble fraction; it may be considered just as well in part as a not absolute insolubility of the eu- fraction in 3.4 saturation ammonium sulphate. The content of agglutinins in the washed euglobulin fraction is of interest, however, as it is more highly "purified" than the pseudoglobulin, so that any relative differences in the distribution of the agglutinins should be from this standpoint the more pronounced for the low fraction.² The limitations in determining the agglutinative potency of the serum and of the fractions, however, make difficult at times the interpretation of the readings obtained, and do not permit conclusions being drawn from a single experiment.

It was found repeatedly in our experiments with rabbit and goat sera that the agglutinins for the dysentery group of organisms (Flexner Manila and Shiga), typhoid, colon and cholera, were not confined

¹ Moll: *Beitr. z. chem. Physiol. u. Path.*, iv, p. 563, 1904.

² The euglobulin of 2 c.c. of serum precipitated at a final dilution of 1:5 was washed usually by being three times thoroughly suspended in 10 c.c. of 3.4 saturation ammonium sulphate solution and recentrifuged.

to either the pseudoglobulin of the washed (with 3.4 saturated ammonium sulphate solution) euglobulin fractions; they were either split by the fractioning, the larger portion occurring in the pseudoglobulin, or almost the entire amount of the agglutinating substances recovered were in this higher fraction in the original quantitative proportion to one another. With antidysentery horse serum, the dysentery (Shiga and Flexner) and *B. coli* agglutinins were fairly quantitatively split between the pseudo- and euglobulin fractions, the latter containing the lesser amount. With an anticholera and anti-typhoid horse serum, the pseudoglobulin (two experiments) and also the filtrates from two additional 3.6 and 3.8 saturation precipitations contained the bulk of both the agglutinins. In subsequent experiments with sera from other bleedings as well as with the sera used above, the typhoid agglutinin was divided between the two fractions with a somewhat larger proportion occurring in the pseudoglobulin.

The results of exhaustion experiments on the two globulin fractions were the same as those that would be obtained in the use of the native serum, and failed to give any reason for believing that we were dealing with a separation of group and specific agglutinins through fractioning.¹

¹ Immunization with certain bacteria results in the development of "group" or "common" and of "specific" agglutinins in the serum of the animal immunized. Group agglutinins are agglutinating substances which are effective both on the homologous organism and on some allied strains of bacteria; specific agglutinin is effective on the organism used for immunization. An immune serum developed by immunization against *B. dysenteriae* (Shiga) might contain, for the sake of illustration, simply (1) a group agglutinin effective for Shiga, Flexner Manila, Pfeiffer and *B. coli*, and (2) an agglutinin specific for the Shiga strain. When immunization has been developed simultaneously against two or three of the above organisms instead of the Shiga strain alone, the number and agglutinating scope of the agglutinins resulting becomes more complex, various group agglutinins and the specific agglutinins for each organism being present. The existence of the two types of agglutinins is demonstrated by the agglutinating characters of the diluted serum after the agglutinins for any desired strain of organism have been exhausted by adding suspensions of washed organisms; after contact for some hours the agglutinated and excess of free organisms are removed by filtration. The agglutinins for several related strains can thus be successively withdrawn. In the example given above, exhaustion with either *B. coli*, Flexner Manila or Pfeiffer would remove only the group agglutinin (1) so that the serum would still agglutinate the Shiga, though at a diminished dilution; exhaustion with the Shiga would take out both the group and the specific agglutinins, and this serum would no longer have agglutinating properties for any of the above organisms. Cf. Castellani: *Zeitschr. f. Hyg. u. Inf.*, xl, p. 1, 1902; also Park and Collins: *Journ. Med. Research*, xii, p. 491, 1904.

Precipitation of antidiphtheria goat serum (three experiments) showed that less than half the antitoxin remained in the pseudoglobulin; practically none was found in the euglobulin while the 3.4 saturated ammonium sulphate solution washings contained the larger part. Our results with the antitoxic horse serum at a dilution of 1:5 are essentially identical with Pick's.

The results of the work accomplished have demonstrated the untrustworthiness of any such differentiation of the antibodies as those contained in the euglobulin and those of the pseudoglobulin. No evidence has been adduced from our experiments to show that the agglutinins developed in the rabbit, goat and horse can be classed as belonging to either globulin, or that these antibodies can be separated from one another by ammonium sulphate fractioning of polyagglutinative sera.¹

A description of the experimental procedure is given with the protocols which follow:

Fractionation of Polyagglutinative Rabbit Serum.

Combined immunization against Flexner Manila dysentery, Shiga dysentery, Pfeiffer,² colon and cholera. Rabbit bled II/27/06. Serum fractioned II/29/06.

Five c.c. of the rabbit serum diluted with 11.5 c.c. of distilled water, were precipitated by 8.5 c.c. of saturated ammonium sulphate solution. After standing two hours, 12.5 c.c. of the uniform mixture were removed and centrifuged in stoppered tubes. The supernatant fluid contains the "pseudoglobulin" fraction. The precipitate ("euglobulin") was washed three times by being thoroughly suspended in 3.4 saturated solution and centrifuged; it was once more suspended and the volume made up to 12.5 c.c. The agglutinating properties were then ascertained of (1) the original serum; (2) the total globulin, a uniform sample of the precipitated serum; (3) the pseudoglobulin fraction, and (4) the washed 3.4 saturation precipitate or euglobulin fraction. Agglutinations were determined microscopically and control slides were ex-

¹ In the paper by Banzhaf and Gibson following this article, it will be shown that the globulins of the serum do differ markedly in their content of antitoxin per gram proteid.

² The original Pfeiffer strain of *B. typhosus*.

amined. Dilutions are in terms corresponding to the original serum. The characters of the agglutinations at the various dilutions are indicated as follows:

- + + + + agglutination with no free organisms.
- + + + agglutination with relatively very few free organisms.
- + + agglutination but with numerous free organisms.
- + incomplete agglutination, small loose groups and many free bacteria.
- ± tendency to agglutinate.
- no agglutination.
- ° observation lost.

The procedure was essentially unchanged in the other experiments. The serum used in this and the following experiments was roughly tested for orientation before the final agglutinations were made. A + + + agglutination indicates usually the highest dilution for an observed position reaction. It should be remembered that the character of the agglutination at any dilution is often difficult to decisively determine; *the actual observations are by no means so exact as would be inferred from the published experiments of Pick and of others.*

The agglutinating properties of this rabbit serum (Table I.), were too low to be entirely satisfactory for fractioning, the weakest agglutinating action (1:50) being manifested on the Shiga strain. The agglutinin for this organism drops out in the euglobulin fraction. This lost agglutinin is not found in the pseudo fraction. It is conceivable from this experiment that the agglutinin of the Shiga dysentery is more soluble than that of the other five strains; however, the Shiga shows no such differences in the two following precipitation experiments on later bleedings of the same rabbit. It is more likely that the content of serum in agglutinin was originally so low that in the fractioned and washed euglobulin the dilution of 1:20 failed to show the proportion of agglutinin present. With the Flexner Manila dysentery, the Pfeiffer typhoid strain, the colon and the cholera—all contained in greater concentration than the Shiga agglutinin—the major portions of the agglutinins occur in the pseudoglobulin, a smaller amount being held by the low fraction.

TABLE I.

Fractioning of Polyagglutinative Rabbit Serum.

Bleeding of II/27/06; fractioning II/29/06.

Organism.	Fraction.	20	50	100	200	500	1000
Flexner Manila.....	Original serum	++++	++++	++++	++++	++	±
	Total gbl.	++++	++++	++++	++++	++	—
	Pseudogbl.	++++	++++	++++	+	±	—
	Eugbl.*	++++	++++	++++	±	—	—
Shiga.....	Serum	++++	++++	++	±	—	—
	Total gbl.	++++	++++	+	—	—	—
	Pseudogbl.	++++	±	—	—	—	—
	Eugbl.*	—	—	—	—	—	—
Pfeiffer.....	Serum	++++	++++	++++	++	±	—
	Total gbl.	++++	++++	++++	++	—	—
	Pseudogbl.	++++	++++	+	±	—	—
	Eugbl.*	++++	++++	+	—	—	—
Colon.....	Serum	++++	++++	++++	++++	±	—
	Total gbl.	++++	++++	++++	++	±	—
	Pseudogbl.	++++	++++	++++	+	±	—
	Eugbl.*	++++	++++	+	—	—	—
Cholera.....	Serum	++++	++++	++++	++++	±	—
	Total gbl.	++++	++++	++++	++++	±	—
	Pseudogbl.	++++	++++	++++	±	—	—
	Eugbl.*	++++	++++	±	—	—	—

* Tested III/1/06 with a fresh culture.

Exhaustion of the two globulin fractions (Table II.) with suspensions of the Flexner Manila strain has not withdrawn the agglutinins for the typhoid from either fraction. Exhaustion with the Pfeiffer likewise is not complete for the dysentery in either pseudo- or euglobulin.

This later bleeding of the same rabbit shows by far the greater part of all the agglutinins in the pseudoglobulin (Table III.), and small though relatively proportional amounts of each in the low fraction. The absolute dropping out of the Shiga does not occur as in the preceding fractioning. The results are also more uniform.

TABLE II.

Exhaustion Experiment, Polyagglutinative Rabbit.

Exhaustion of the Original Serum and the Fractions (cf. Table I.).

Exhaustion with Flexner Manila.

Organism.	Fraction.	20	50
Flexner Manila.....	Serum	+	—
	Pseudogbl.	—	—
	Eugbl.	—	—
Pfeiffer.....	Serum	++++	++++
	Pseudogbl.	++++	++++
	Eugbl.	++++	++++

Exhaustion with Pfeiffer.

Flexner Manila.....	Pseudogbl.	++++	—
	Eugbl.	++++	—
Pfeiffer.....	Pseudogbl.	—	—
	Eugbl.	—	—

TABLE III.

Fractioning of Polyagglutinative Rabbit Serum.

Serum from bleeding IV/16/06; serum fractioned IV/18/06.

Organism.	Fraction.	50	100	200	500	1000	2000
Flexner Manila.....	Total gbl.	++++	++++	++++	++++	++++	+
	Pseudogbl.	++++	++++	++++	++++	++	+
	Eugbl.	++	+	—	—	—	—
Shiga.....	Total gbl.	++++	++++	++++	++++	++++	++
	Pseudogbl.	++++	++++	++++	++++	++	±
	Eugbl.	++	+	—	—	—	—
Cholera.....	Total gbl.	++++	++++	++++	++++	++++	++
	Pseudogbl.	++++	++++	++++	++++	++++	+
	Eugbl.	+	—	—	—	—	—
Pfeiffer	Total gbl.	++++	++++	++++	++++	++	+
	Pseudogbl.	++++	++++	++++	++++	+	±
	Eugbl.	±	—	—	—	—	—

The third precipitation (Table IV.), shows apparently a recovery of all the agglutinins in the high globulin fraction. The pseudoglobulin dilutions for the Flexner Manila, in fact, were read slightly higher than were those of the total globulin.

Pick's single observation by the test-tube method on typhoid rabbit serum, agglutinating at 1:20,000, gave the limit of agglutination of the pseudoglobulin at 1:3000, of the euglobulin 1:20,000; after reprecipitating the fractions three and four times, respectively, the limits of dilution for agglutination were at 1:20 and 1:8000.

TABLE IV.
Fractioning of Polyagglutinative Rabbit Serum.
Serum from bleeding on V/23/06.

Organism.	Fraction.	50	100	200	500	1000
Flexner Manila.....	Total gbl.	++++	++++	++++	++++	+++
	Pseudogbl.	++++	++++	++++	++++	++++
	Eugbl.	—	—	—	—	—
Pfeiffer.....	Total gbl.	++++	++++	++++	++++	+++
	Pseudogbl.	++++	++++	++++	++++	+++
	Eugbl.	—	—	—	—	—
Cholera.....	Total gbl.	++++	++++	++++	+++	++
	Pseudogbl.	++++	++++	++++	+++	++
	Eugbl.	±	±	±	—	—

Polyagglutinative Goat Serum.

Immunization against Flexner Manila, Shiga, Pfeiffer, cholera and *B. coli*.

The proportion of the agglutinins for each organism is much greater in the pseudoglobulin than in the euglobulin fractions (Table V.). This conclusion is confirmed for the Shiga, Pfeiffer and cholera by the redetermination of the agglutinations in the exhaustion experiment (Table VI.). The Flexner Manila dysentery strain has not exhausted the agglutinins for the other organisms completely from the eu- or from the pseudoglobulin fractions; nor have the agglutinins apparently been

withdrawn to a relatively greater degree from the one than from the other fraction.

Fractioning of the polyagglutinative serum (of lessened agglutinating power) from the second bleeding of the goat immunized against the mixed cultures showed that the agglutinins were almost quantitatively contained in the pseudoglobulin fraction (Table VII.).

TABLE V.

Fractioning of Polyagglutinative Goat Serum.

Serum from bleeding II/27/06; fractioned III/9/06.

Organism.	Fraction.	50	100	200	500	1000
Flexner Manila	Serum	++++	++++	++++	+++	++
	Total gbl.	++++	++++	++++	+++	+
	Pseudogbl.	++++	++++	+++	++	—
	Eugbl.	++++	+++	—		
Shiga	Serum	++++	++++	++++	++++	++
	Total gbl.	++++	++++	++++	++++	+
	Pseudogbl.	++++	++++	++++	++++	—
	Eugbl.	++++	+++	—		
Pfeiffer	Serum	++++	++++	++	++	+
	Total gbl.	++++	++++	+++	+++	±
	Pseudogbl.	++++	+++	++	+	±
	Eugbl.	+++	+	—		
Cholera	Serum	++++	++++	++++	++++	+++
	Total gbl.	++++	++++	++++	++++	+++
	Pseudogbl.	++++	++++	+++	++	±
	Eugbl.	++	+	+		
Colon.	Serum	++++	++++	++++	++++	+++
	Total gbl.	++++	++++	++++	+++	+
	Pseudogbl.	++++	++++	++++	+++	±
	Eugbl.	+++	++	—		

TABLE VI.

Exhaustion Experiment. Polyagglutinative Goat Serum.

Exhaustion with Flexner Manila of Fractions (Table V) on III/7/06.

Fraction.	Organism.	20	50	100	200	500	1000
Total gbl	Flexner Manila	++++	—	—	—	—	—
	Shiga	++++	++++	++++	++++	++++	+++
	Pfeiffer	++++	++++	++++	++++	++++	+++
	Cholera	++++	++++	++++	++++	++++	++++
Pseudogbl	Flexner Manila	—	—	—	—	—	—
	Shiga	++++	++++	++++	++++	++	
	Pfeiffer	++++	++++	++++	++++	+	
	Cholera	++++	++++	++++	++++	+++	
Eugbl.	Flexner Manila	—	—	—	—	—	
	Shiga	++++	+++	+	—	—	
	Pfeiffer	++++	+++	—	—	—	
	Cholera	+	+	—	—	—	

Pick's corresponding experiment may be summed up at follows: As antityphoid goat serum agglutinating at 1:2600 was precipitated in a series of progressively increasing concentrations of ammonium sulphate. The initial precipitation was at 2.6 saturation with a dilution of 1:5, agglutination being evident at 1:500 of the unfiltered precipitated mixture. At 3.4 saturation over half and at 3.6 saturation all the agglutinin was precipitated. Again, 20 c.c. of the same serum were precipitated with 10 c.c. of saturated ammonium sulphate solution; the euglobulin then agglutinated at 1:2400 (in terms of original serum) the pseudoglobulin at 1:20. After two reprecipitations the eu-fraction still reacted at 1:2400.

TABLE VII.

Fractioning of Polyagglutinative Goat Serum.

Bleeding of III/18/06; fractioned III/20/06.

Organism.	Fraction.	100	200	500	1000
Flexner Manila	Total gbl.	++++	++++	+++	±
	Pseudogbl.	++++	++++	++	—
Shiga	Total gbl.	++++	++++	o	—
	Pseudogbl.	++++	++++	++++	±
Pfeiffer.....	Total gbl.	++++	+++	±	—
	Pseudogbl.	++++	++++	—	—
Cholera	Total gbl.	++++	++++	+	—
	Pseudogbl.	++++	++++	+	—
Colon	Total gbl.	++++	+++	++	—
	Pseudogbl.	++++	+++	—	—

Polyagglutinative Horse Serum.

(a) *Antidysentery Horse Serum.* Horse 284; immunized against the Shiga, Flexner Manila and the Mount Desert strains. Bleeding of X/3/06; fractioned X/4/06.

Here (Table VIII.), the agglutinins are split between the fractions, the larger part of each occurring in the pseudoglobulin.

(b) *Anticholera and Antityphoid Horse Serum.* Horse 254; combined immunization against the original Pfeiffer and cholera. Serum from bleeding on III/27/06; fractioned IV/1/06 and refractioned V/10/06. The results are given in Table IX.

With the cholera-typhoid serum, the agglutination values of which for each organism were in the neighborhood of a dilution of 1:1000, the bulk of the agglutinins was found in the pseudoglobulin (Table IX.); the greater portion was also in the high fraction when the serum was precipitated at 3.6 and again at 3.8 saturation. There is no evidence presented here that the precipitation limits of the cholera agglutinin are in any way different from that of the typhoid. In the second fractionation (V/10/06) it is seen that both the Pfeiffer and the cholera are increased in the eu-fraction as contrasted with the result of the first precipitation at 3.4 saturation.

TABLE VIII.

Fractioning of Antidystentery Horse Serum.

Organism.	Fraction.	50	100	200	500	1000	2000
Flexner Manila.....	Serum	++++	++++	++++	++++	+++	++
	Total gbl.	++++	++++	++++	+++	+	+
	Pseudogbl.	++++	++++	+++	+++	+	—
	Eugbl.	++++	++++	+++	±	—	—
Shiga	Serum	++++	++++	++++	++++	+++	—
	Total gbl	++++	++++	++++	++++	±	—
	Pseudogbl.	++++	++++	++++	—	—	—
	Eugbl.	++++	++++	+++	—	—	—
Colon.....	Serum	++++	++++	++++	++++	++++	o
	Total gbl.	++++	++++	++++	+	—	—
	Pseudogbl.	++++	++++	++++	±	—	—
	Eugbl.	++++	+++	++	±	—	—

The fractionation of the anticholera and antityphoid horse serum is of especial interest because an experiment of this nature is the most striking of E. P. Pick's observations. Pick had found that the typhoid agglutinin was precipitated with the pseudoglobulin fraction in horse serum; the cholera agglutinin, on the contrary, came down with the euglobulin. Pick, therefore, mixed equal volumes of a typhoid and a cholera serum, and progressively precipitated 2 c.c. amounts of the mixed sera at 2.8, 3.0, 3.2, etc., saturation (with a final volume of 10 c.c. in each case). A well marked separation of the cholera agglutinin into the euglobulin and the typhoid agglutinin into the high fraction resulted. As given in Pick's tables, the observed agglutination values are twice too much, since each serum was diluted a half by the mixing. A direct separation at 3.4 saturation of the agglutinin in a goat cholera serum (agglutinating at a dilution of 1:1000) from the typhoid agglutinin in horse serum (1:20,000) was made. The euglobulin was precipitated by adding a half volume of saturated ammonium sulphate solution directly to the mixed sera. The high agglutination values are again given as *direct* observations, though probably calculated for the original undiluted sera. The figures for the reprecipitated euglobulin

are for the typhoid, 1:3000, and for the cholera 1:1600 (an increase over the value of the original goat serum); the pseudoglobulin (3.3 saturation filtrate), agglutinated the typhoid at 1:16,000, the cholera at 1:20.

TABLE IX.
Fractioning of Anticholera-Antityphoid Horse Serum.

1. Precipitation at 3.4 saturation.

Organism.	Fraction.	50	100	200	500	1000
Pfeiffer.....	Total gbl.	++++	++++	++++	++++	++++
	Pseudogbl.	++++	++++	++++	+++	±
	Eugbl.	+	+	—	—	—
Cholera.....	Total gbl.	++++	++++	++++	++++	++++
	Pseudogbl.	++++	++++	+++	+++	+
	Eugbl.	+	+	—	—	—

2. Precipitation at 3.6 saturation.

Organism.	Fraction.	50	100	200	500	1000
Pfeiffer.....	Total gbl.	++++	++++	++++	++++	++
	Pseudogbl.	++++	++++	++++	+++	—
Cholera.....	Total gbl.	++++	++++	++++	++++	++++
	Pseudogbl.	++++	++++	++++	++++	++++

3. Precipitation at 3.8 saturation.

Organism.	Fraction.	50	100	200	500	1000
Pfeiffer.....	Total gbl.	++++	++++	++++	++++	±
	Pseudogbl.	++++	++++	+++	+++	±
Cholera.....	Total gbl.	++++	++++	++++	++++	++++
	Pseudogbl.	++++	++++	++++	+++	±

4. The same serum was again fractioned on V/10/06.

Organism.	Fraction.	50	100	200	500	1000	2000
Pfeiffer	Total gbl.	++++	++++	++++	++++		
	Pseudogbl.	++++	++++	++++	+++		
	Eugbl.	++++	++++	++++	—		
Pfeiffer*	Total gbl.	++++	++++	++++	++++	+++	++
	Pseudogbl.	++++	++++	++++	+++	±	—
	Eugbl.	++++	++++	—	—	—	—
Cholera.....	Total gbl.	++++	++++	++++	++++	+++	
	Pseudogbl.	++++	++++	++++	+++	++	
	Eugbl.	++++	++++	+++	±	—	

* A second fractionation.

TABLE X.

Fractioning of Anticholera-Antityphoid Horse Serum.

Horse 254; bleedings of II/27/06 and V/29/06; fractioned X/8/06.

Fractions tested with the Mt. Sinai culture of typhoid.

Centrifuged.	Fraction.	100	200	500	1000	2000
2 hrs. after precipitation.....	II/27/06					
	Total gbl.	++++	++++	++++	+++	+
	Pseudogbl.	++++	+++	+++	±	
After 12 hrs.....	Eugbl.	++++	+++	+	±	
	Total gbl.	++++	++++	++++	++++	+++
	Pseudogbl.	++++	+++	+++	±	
After 2 hrs.....	Eugbl.	++++	+++	+	±	
	V/29/06					
	Serum	++++	++++	++++	+++	+
After 2 hrs.....	Total gbl.	++++	++++	++++	+++	+
	Pseudogbl.	++++	++++	+++	++	±
	Eugbl.	++++	++++	+++	++	±
After 12 hrs.....	Total gbl.	++++	++++	++++	+++	+
	Pseudogbl.	++++	+++	+++	+	
	Eugbl.	+++	++	±		

In the following observations it is shown that a relatively large proportion of the typhoid agglutinin may occur in the euglobulin fraction.¹

Six c.c. of each serum were diluted with 13.8 c.c. of water and precipitated by the gradual addition of 10.2 c.c. of saturated ammonium sulphate solution. Uniform samples (15 c.c.) of each were centrifuged after two hours' standing. The euglobulin precipitates were washed by thoroughly suspending the proteid in 15 c.c. of 3.4 saturation ammonium sulphate solution and again centrifuging; the precipitates were washed three times in this fashion. Exactly similar precipitations were made on the two sera, but the precipitated mixtures were centrifuged after twelve instead of two hours' standing. The results are given in Table X.

A high typhoid agglutinin content in the euglobulin was also obtained on fractioning the last bleeding of the cholera-typhoid horse. The results are shown in Table XI.

TABLE XI.

Fractioning of anticholera-antityphoid Horse Serum.

Horse 254; bled X/3/06; fractioned X/4/06; tested only with typhoid.*

Organism.	Fraction.	100	200	500	1000	2000
Mt. Sinai Typhoid.....	Total gbl.	++++	++++	++++	++++	++
	Pseudogbl.	++++	++++	++++	+++	—
	Eugbl.	++++	++++	+++	—	—
St. Typhoid.....	Total gbl.	++++	++++	++++	+	+
	Pseudogbl.	++++	++++	++++	++	—
	Eugbl.	++++	++++	+++	—	—

* The Pfeiffer strain agglutinated spontaneously. The Mount Sinai strain has always shown the same agglutinations as the Pfeiffer in numerous other observations.

Fractionation of Diphtheria Antitoxic Goat and Horse Serum.

Fresh goat serum and serum from horse 307, VII/5/06, were fractioned as follows: 3 c.c. of serum diluted with 6.9 c.c. of water were precipitated with 5.1 c.c. of saturated ammonium sulphate solution, and

¹On resuming this problem in the fall of the year, it was found that our cholera culture was spontaneously agglutinating; it could not therefore be employed in testing the agglutination values of the fractions.

the precipitate from a 10 c.c. sample of each obtained by centrifuging. The precipitates were suspended in 3 c.c. of 3.4 saturated ammonium sulphate solution and again centrifuged; the washing was twice repeated and the wash solutions united and made up to 10 c.c. The precipitates were suspended as usual in 3.4 saturated ammonium sulphate and made up to 10 c.c. The results calculated per c.c. of the original undiluted serum follow:

Fraction.	Goat.	Horse.
Serum	90 units	250 units
Total globulin.....	90 "	250 "
Pseudoglobulin.....	35 "	+200 "
Euglobulin.....	5 "	0* "
Wash solution	50 "	+25 "

* Tested for 5 units against 100 m. l. d.; the guinea pig died in 12 hours, autopsy showing a typical diphtheria toxin picture.

The same results were obtained in two similar experiments. The two sera certainly show a different behavior towards ammonium sulphate precipitation. A relatively large proportion of the goat antitoxin is precipitated with the euglobulin. The facility with which the antitoxin can be washed out almost completely (in a total united volume of wash solution less than the original volume of the precipitated mixture) shows that the antitoxin is not invariably linked to the euglobulin.

Pick's experiment is given briefly for comparison with our results:

Twenty c.c. of antitoxic goat serum¹ (neutral reaction) were precipitated with 10 c.c. of saturated ammonium sulphate solution. After two hours, the pre-

¹ Pick states (p. 361) "Zu dem nun folgenden Trennungsversuche mit Diphtherie immun Ziegenserum stand mir nur ein Ziegenserum zur Verfügung, von dem 0.1 c.cm. eben im Stande war, die 10-fache tödliche Giftmenge eines Toxins zu paralysieren das in der Dosis von 0.0098 c.cm. ein Meerschweinchen von etwa 260 g. in drei Tagen tötete." This would make the potency of the serum used only 1 unit per c.c. The control test made actually gives the strength as 10 antitoxin units per c.c.: 0.0098 c.c. toxin (10 m.l.d.) were neutralized by 0.01 c.c. serum; therefore 100 m.l.d. toxin were neutralized by 0.1 c.c., or 1 c.c. serum neutralized 10 × 100 m.l.d. toxin. Ledingham has passed this over: "The goat serum with which Pick worked had a very low antitoxic value inasmuch as 0.1 c.c. was required to neutralize 10 lethal doses of a toxin whose m.l.d. was only about 0.01 c.c." Pick, himself, speaks incidentally "Man erkennt trotz der geringen antitoxinischen Wertes von diesem Serum"

precipitate was pressed out and dissolved in 30 c.c. of water; it was reprecipitated at 3.3 saturation and dissolved in 20 c.c. of water (euglobulin fraction). The filtrates were united and made up to half saturation, the precipitate dissolved in the original volume (20 c.c.) and refractioned between 3.3 and 5.0. The euglobulin then obtained was united with the above euglobulin solution and the mixture precipitated at 3.3 saturation. The serum contained about 10 units per c.c. The fractions tested as follows:

Pseudoglobulin:

0.05 c.c. + 10 m.l.d. toxin (testing for 2 units). The guinea pig died on the second day.

Euglobulin:

0.01 c.c. + 10 m.l.d. toxin (testing for 10 units). Died on the third day.

0.017 c.c. + 10 m.l.d. toxin (testing for 6 units). Induration and loss of weight, but survived.

Ledingham states in his conclusions that in the horse serum the relationship of the diphtheria antitoxin to the pseudoglobulin fraction "holds good only when the antitoxin content of the serum is steadily rising." Horse 307 of this department had been subjected to immunization for over five months; it attained a maximum of over 300 units per c.c. in three months and had declined to 250 units two months later when the blood of the serum used in our fractionation experiments was drawn.¹ Apparently Ledingham's conclusion (from observations on a single horse) is not of general application.

Tabulating Pick's results by a somewhat different arrangement than the one presented in his paper (p. 384), it is seen from the following—

Animal.	Diphtheria Antitoxin.	Tetanus Antitoxin.	Cholera Lysin (Pfeiffer).	Typhoid Agglutinin.	Cholera Agglutinin.
Goat	eugbl.	eugbl.	eugbl.	eugbl.	eugbl.
Rabbit				eugbl.	
Guinea pig				eugbl.	
Horse	pseudogbl.	pseudogbl.		pseudogbl.	eugbl.

—that there is no evidence of any differences in the precipitation limits of the antibodies in goat, rabbit and guinea pig sera. We should hardly expect *a priori*, then, that a separation of any other antibodies by fractionation of goat and rabbit serum would be possible, and we have not

¹ The horse subsequently was killed as no longer of service for antitoxin production.

found otherwise. Yet the probability of such a separation for horse serum was suggested by Pick's experiments. With this serum, even, the distribution of the antibodies as determined by Pick, has been similarly homogeneous with the exception of the cholera agglutinin. Pick, accordingly, gives one example, and one only, of an antibody differing from other antibodies in the serum of the same species by its precipitation characters toward ammonium sulphate. This observation of Pick's we have been unable to verify when polyagglutinative horse sera have been used. It is probable, however, that the serum globulins of different animals or even of various individuals of the same species may show a different and inconstant behavior quantitatively toward fractional ammonium sulphate precipitation. We have not found that any of the antibodies in goat, rabbit and horse serum were invariably associated with the euglobulin. Our results with goat diphtheria antitoxin have been confirmed by Ledingham. At the same time we have presented repeated observations with several strains of typhoid showing that a large proportion (almost half in some instances) of the typhoid agglutinin of horse serum may be found in the thoroughly washed euglobulin fraction of both old and fresh serum.

The results of our experiments have already been briefly summarized in the preceding portion of the paper.

It is to be hoped that any future work on the fractionation of the antibodies or of the proteids of the blood will not be undertaken without a thorough comprehension of the nature and limitations of the process. Salt fractionation is a valuable method for the purification and preparation of proteid products; the salt concentration precipitation limits, however, are not a reliable means for differently classifying proteids the precipitation characters of which are not widely separated.

THE PRODUCTION OF AGGLUTININS IN THE ANIMAL BODY BY THE INOCULATION OF SUBSTANCES OTHER THAN PRODUCTS OF BACTERIAL ORIGIN.*

By KATHARINE R. COLLINS, M. D.

There is to-day among observers working along the lines of immunity a tendency to no longer consider a given anti-body and the factors producing it as a compact inseparable entity following the laws of protoplasmic continuity, but as a complex body that may be split up into component parts, which parts may exhibit individuality of structure and independence of action. This view of the subject has been given an impetus by the work of Vaughan and later Obermeyer and Pick. Vaughan derived split products from bacterial proteids and egg-albumen, representing a poisonous and non-poisonous portion. Obermeyer and Pick found by iodizing protein they so changed it that when the iodized portion was inoculated into animals only non-specific precipitins were formed. This led them to believe that specificity in this case was due to the aromatic radical which was changed by iodization.

The following work was begun in 1905 with hope of determining through the nature of substances having the power to produce agglutinins in the animal a more intimate knowledge of the anti-bodies and their elaboration in the animal economy. This in a measure was realized. As the work progressed the fact developed that, as far as tested, certain molecules or radicals containing such molecules, are always present in the substances, which induce an increase of agglutinin production; this fact strongly suggests the possibility that these molecules are responsible, for one feature at least, of this phenomenon. The observations arrange themselves under the following heads: (1) organized ferments, (2) unorganized ferments, (3) metabolic products, (4) putrefactive products, (5) inorganic substances.

Organized Ferments—Ballner and Sagasser, in 1904, succeeded in producing, artificially, agglutinins for *Bacillus typhosus* by inoculating animals with red yeast cells (Rosa Hefe), but the yeast cells themselves were not agglutinated by the serum.

* Read before the American Association of Pathologists and Bacteriologists, April 17, 1908.

I have not been able to identify the Rosa Hefe used by Ballner and Sagasser. The red yeasts mentioned in most works on fermentation is, strictly speaking, a torula and not a true yeast. Several rabbits were inoculated with the cells of the so-called red yeast obtained from the air. This did not give rise to an increase in the production of agglutinins, though later these same rabbits responded to inoculation of brewer's yeast cells. Acting upon this suggestion of Ballner, I inoculated rabbits with living cultures of brewer's yeast. After four or five inoculations the sera of these rabbits were tested with the following organisms:

1. *B. dysenteriae* (3 strains), Shiga, Flexner Manila, Park Mt. Desert.
2. *B. typhosus* (2 strains), Pfeiffer, Mt. Sinai.
3. *B. Coli* (2 strains) (Laboratory, Colon X.).
4. *B. paratyphosus*.
5. *B. pyocyaneus*.
6. *B. proteus vulgaris*.
7. *S. cholerae*.
8. *Pneumococcus*, one strain.
9. *Streptococcus*, one strain.
10. *B. mallei*.

The only organisms that reacted with the various immune sera were Flexner Manila—*B. typhosus*, Pfeiffer strain and Colon X. Several strains of brewer's yeast, differing in their action upon beerwort, were tested without presenting any appreciable variation in results. The brewer's yeast was obtained in pure cultures from the laboratory of the Brewer's Academy, New York City, and were cultivated on 10 per cent. beerwort agar. Living cells were used. Beerwort alone inoculated into rabbits was without effect.

The result of inoculations with yeast cells may be shown in the following table:

TABLE I.

	Normal Serum.			After 8 Inoculations.					
	10	20	50	10	20	50	100	200	500
Shiga.....	—	—		—	—				
Flexner Manila.....	+	+	—	++	++	++	++		
Park Mt. Desert.....	—	—		—	—	—			
Pfeiffer.....	+	—		++	++	++	++		
Colon X.....	+	—		—	—				

	After 13 Inoculations.						After 18 Inoculations.						
	10	20	50	100	200	300	10	20	50	100	200	500	1000
Shiga.....	—						—						
Flexner Manila.	++	++	++	++	++	+	++	++	++	++	++	++	++
Park Mt. Desert		---	—	—	—		—	—					
Pfeiffer.....	+	+					—						
Colon X.....	—						—						

Several rabbits inoculated with yeast cells also gave the same results as the goat. The serum of these rabbits, when tested, showed a steady increase after inoculation, for the Flexner Manila strain of *B. dysenteriae*, an increase for *B. typhosus* and sometimes Colon X. strain *B. coli* with the subsequent disappearance of the two latter. This disappearance of the agglutinins for *B. typhosus* and Colon X. would seem to indicate one of two things. First, the normal cells of the rabbit possess a greater potentiality for the manufacture of Flexner Manila agglutinins, on account of the cell being subjected to a stronger and more specific stimulation in this direction and therefore when influenced by non-specific substances the cell forms agglutinins for this organism in preference to others because of this stronger initial stimulation. As the inoculations proceed the increment of production for the Flexner Manila strain becomes more marked and finally the entire energies of the cell seem to be occupied in forming these agglutinins to the exclusion of the others.

On the other hand the possibility suggests itself that the stimulating agent may not be so entirely non-specific as generally considered. This phase would then agree with that seen when definite specific organized agents such as bacteria or their products are used, stimulation of common agglutinins being induced. Common agglutinins may continue throughout to be formed to an equal degree along with the specific, or may disappear almost entirely after long immunization. This disappearance or persistence seems to depend somewhat upon the relation of the heterologous organisms to the homologous. The more nearly the species are related the more persistent the common agglutinins.

The serum of a rabbit immunized with yeast cells was subjected to the exhaustion test with *B. typhosus*, the Flexner Manila organism and living brewer's yeast cells—and cells of the torula. The agglutinins for Flexner Manila were completely exhausted by this organism, *B. typhosus*, and the yeast cells absorbed all but 10 per cent. of the Flexner Manila agglutinins. This amount not absorbed approximately represents the amount of normal agglutinins for Flexner Manila present in the serum, which, on account of its more specific nature, would resist the action of purely non-specific agents.

Unorganized Ferments—The possibility that other enzymes might bring about this increase in the animal of pre-existing agglutinins suggested the use of the unorganized ferments, diastase, pancreatin and invertin. The results obtained by the inoculation of these substances coincide with those obtained by the inoculation of yeast cells.

The agglutinins were in each case increased from 1:50 to 1:500 after five or six, weekly, inoculations. The enzymes, however, failed to absorb the agglutinins thus raised from the homologous serum.

Products of Metabolism—Nuclein as a component part of the yeast cell was first used. Vaughan obtained a certain amount of protection against the pneumococcus in guinea pigs by the inoculation of nuclein previous to the pneumococcus infection. The nuclein was used in the form of a nucleoproteid from the pancreas, for which I am indebted to Dr. Levene, of the Rockefeller Institute for Medical Research. The other products used of the metabolic group were lecithin and proteoses from egg. The same increase of agglutinins for Flexner Manila type of *B. dysenteriae* followed the inoculation of these substances a

with yeast and enzymes. There was initial rise for Flexner Manila type, *B. typhosus* and *B. coli*, with the subsequent dropping out of the two latter and a continued rise of the former as the inoculations were continued.

Products of Putrefaction—The products of putrefaction tested were indol, skatol, ethyl mercaptan and phenol. I am indebted to Dr. C. A. Herter for the indol, skatol and mercaptan, and also for some valuable suggestions as to their use.

Ethyl mercaptan in one per cent. solution produced an increase of agglutinins in two rabbits after the fifth inoculation and followed the same course as in the cases where the enzymes and metabolic products were used. A control rabbit on injection of ethyl alcohol did not show an increased production of its normal agglutinins. Indol and skatol were without effect, as might have been expected on account of the readiness with which they combined with the preformed sulphates and were excreted. Phenol, however, did enter into systemic relation with the organism and toxic effects were demonstrated in the animals inoculated, but a rise of agglutinins did not occur after a number of inoculations, the animals finally dying from excessive abscess formation.

At this point two factors present themselves as possible influences in causing an increase in agglutinins. The first is the increased production and destruction of leucocytes and the second is the fact that the substances bringing about this increase of agglutinins with the exception of the enzymes, concerning whose structures little is known, all possess a formula containing phosphorus or sulphur molecules, while those failing to effect an increase do not possess either elements.

First the effect of the inoculations of these substances upon the leucocytes may be considered. Dieudonne claims that animals possessing agglutinins for a certain organism may have the amount of agglutinins increased by the inoculation of excitors of leucocytes, such as aleuronat and hetol. Aleuronat in our hands brought about an increase of the initial agglutinins similar to that caused by the substances used in the preceding experiments. Hetol was not used.

To test the effect of our inoculations upon leucocytes, blood counts were made upon normal rabbits, rabbits inoculated with substances increasing the agglutinins, and substances which did not effect the ag-

glutinins. The blood counts exhibit the same irregularity in the normal rabbit and in the inoculated rabbits irrespective of the occurrence or non-occurrence of an increase in agglutinins. This irregularity is in accordance with the statement of Brinckerhoff that the number of leucocytes per millimeter in the peripleral blood of the rabbit is constantly changing. It would appear from this irregularity that leucocytes could not account for the increased production. The action of aleuronat should, therefore, be ascribed to its behavior as a proteid rather than as an excitor of leucocytosis.

Inorganic Salts—To follow more in detail the suggestion developed by the fact that phosphorus and sulphur were the elements possessed in common by the augmentors of agglutinins, several soluble inorganic salts of sulphur and phosphorus were tested, and their action was controlled as far as practical by salts containing the same base but not the same radical.

Several rabbits were inoculated with sodium phosphate, sodium sulphate, calcium and potassium phosphate. Control rabbits were inoculated with sodium chloride, calcium chloride, and potassium chlorate. A young goat was inoculated with sodium sulphate and a control goat with sodium chloride. The sera of the rabbits and the goat receiving the sulphur and phosphorus compounds showed the characteristic increase of agglutinins for the Flexner Manila strain of *B. dysenteriae*. The agglutinins for *B. typhosus* and *B. coli* were only slightly stimulated in a few instances. The agglutinins of the control animals inoculated with the salts that did not contain the sulphur or phosphorus molecules were unaffected. The doses varied according to the toxicity of the salts used and the concentrations ranged from one-tenth normal to twenty-five per cent. solutions. The concentration of the solution had no apparent effect upon the results. It is interesting to note that Vaughan found the phosphorus in the non-toxic portion of the split products to be the part which gives immunity.

A question arises as to the character of the action of these substances. Do they merely stimulate to greater activity a specific function of the cell already established, or do they possess something in common with the bacteria which admits of their initiating specific action in

some degree? An attempt was made to answer this question in the following manner:

Two rabbits each having normal agglutinins for Flexner Manila type of *B. dysenteriae* up to 1:50, but none that were appreciable for Shiga or Park Mt. Desert types, were inoculated with the Shiga type and the other with the Park Mt. Desert organism. After three inoculations the sera of these rabbits agglutinated their homologous organism in dilutions of 1:100. The index for Flexner Manila type remained unchanged. The bacterial inoculations were then stopped and sodium sulphate substituted in one rabbit and diastase in the other. After four or five treatments the sera from both rabbits were tested. The agglutinins for the Flexner Manila organism were increased up to 1:500, while those for Shiga and Park Mt. Desert type remained unchanged and later disappeared.

These two experiments are not sufficient, however, to prove or disprove the assumption that the action is one of augmentation and not of initiation. I have mentioned the foregoing question because it suggests several points to be taken into consideration in answering it. First, the influence that has brought about the normal agglutinins in the rabbit for Flexner Manila has been acting practically during the adult life of the animal, while the influence of the Shiga or Park Mt. Desert organisms has only been exerted for a comparatively short period of time. Hence the function of the cell to form the Flexner Manila agglutinins would be more permanent than for the other two organisms. Now, when the rabbits are inoculated with strong specific substances as Shiga or Park Mt. Desert, the cell responds accordingly as long as this stimulus is kept up. But upon removal of this influence and the substitution of a new, presumably, specific stimulus, then the cell by preference responds in the direction of the more accustomed function of forming agglutinins for Flexner Manila. Another point to be considered is the fact that after the withdrawal of the specific influences of Shiga and Park Mt. Desert the cause producing the normal agglutinins for Flexner Manila in the rabbit continues to act, thus adding its influence to the stimulus of the non-specific sodium sulphate inoculations and so determining the direction of the activity of the cells.

At this stage of the present investigation biological rather than physical laws seem to offer the most probable explanations of the production or augmentation of agglutinins which has been described; perhaps stimulation of certain cell activities occur because some necessary element which enters into the cell or acts as a ferment adjuvant is provided.

The work of several authors on ferments is suggestive by analogy. Bertrand found that manganese was present in laccase and activity of laccase was proportional to the amount of this salt present.

Calcium salts are found to be essential to enzymes which cause clotting and Magnus has given the name of co-ferments to those substances, but this term has been rejected by Hardin and Young. Hardin and Young found that boiled yeast which was capable of initiating fermentation alone when added to unboiled yeast increased its action to a considerable extent. They found this increase due to the presence of soluble phosphates. Arsenates brought about the same results as the phosphates.

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IS THE PRESENT METHOD OF STANDARDIZING ANTI-DIPHTHERIC SERUM ACCORDING TO ANTI-TOXIN UNITS THERAPEUTICALLY ACCURATE?

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Cruveilhier¹ quotes Roux, Marfan, Martin and Momont, as finding that the dose of antidiphtheric serum most efficacious therapeutically is not always the one which contains the greatest number of antitoxic units. These opinions are contrary to those formed by the physicians observing the cases treated in the hospitals in New York City. The experiences of these authors, Cruveilhier says, seems to indicate that the serum contains, besides the antitoxin, other important preventive and curative substances. In the standardization of antidiphtheric serum as at present practiced, namely, according to antitoxic units, these protective substances are entirely ignored. Cruveilhier to test this point, carried out a series of experiments with guinea pigs, infected with diphtheria bacilli. He compared sera of different antitoxic strength from several horses as to their value preventively and curatively. In his preventive experiments he injected subcutaneously a quantity of serum proportional to the weight of the animal; and, 24 hours later, inoculated subcutaneously a fatal dose of diphtheria culture. He reported the following results: In four out of seven experiments, with culture No. 261, the animals which received 1/250,000 of 1 c.c., per gram weight, of a 200-unit serum resisted; while those which received the same volume quantity of a 500-unit serum died. In only one instance did the 500-unit serum prove superior, per volume quantity, to the 200-unit serum. Twice the results obtained per volume quantity for both sera were the same. On comparing a 50-unit with one of 500 units, the 50-unit serum proved superior to the 500-unit one, per volume quantity.

With two other cultures, designated "c.c." and "x," he compared a 200-unit serum with a 500-unit one, and obtained the same results.

¹*Ann. de l'Inst. Past.*, 1904, 18, p. 249.

Again, using culture No. 261, he compared a 300-unit serum with one of 500 units, from different bleedings of the same horse, and found them equal per volume in protection.

In his curative experiments, using the same cultures as in the preventive, he injected guinea-pigs subcutaneously with that amount of diphtheria culture which killed the control animal in from 36–48 hours. The animals were divided into two lots. At intervals of two hours, from the second to the sixteenth hour after inoculation, each animal of one lot received subcutaneously 0.1 c.c. of the serum of the lesser unit content, while each animal of the second lot received 0.1 c.c. of the serum of a greater unit content. In his curative experiments the sera of the lesser unit content were more efficacious than those of the greater.

These results apparently showed that it was the quantity of serum, rather than the number of antitoxic units, which was of therapeutic value.

Cruveilhier drew the following conclusions:

“Que l'effet curatif d'un sérum ne dépend pas exclusivement de sa teneur en unités antitoxiques.

“Que le titrage de l'antitoxine, tel qu'on le pratique habituellement, ne suffit pas à rendre un compte exact de l'efficacité d'un sérum. Que celle-ci est plus exactement appréciée parce que nous avons appelé la mesure du pouvoir thérapeutique.”

In view of the large amount of work which has been done on the titration of diphtheria antitoxin, the results of Cruveilhier cannot but be considered extraordinary.

We have carefully investigated this subject and have obtained results diametrically opposed to those of Cruveilhier and the authors he cites.

In our experiments we compared both preventively and curatively, native sera and antitoxic globulin solutions (Gibson¹) of the following unit content: A serum of 43 units compared with an antitoxic globulin solution of 1,700 units; a serum of 43 units with an antitoxic globulin solution of 1,450 units; a serum of 200 units with one of 1,000 units;

¹ *Jour. Biol. Chem.*, 1906, p. 161.

a serum of 600 units with one of 1,300 units; a serum of 600 units with one of 335 units, also with one of 200 units. The last three sera were obtained from the same horse during the course of immunization.

The animals infected were active, healthy normal guinea-pigs weighing between 240 and 260 grams.

The diphtheria bacilli were from the three following strains: Culture No. 1, a moderate toxin producer; culture No. 2, a weak toxin producer. Both of these were freshly isolated from the throats of diphtheria patients at the Department of Health Hospital. Culture No. 8 (Park and Williams), is a strong toxin producer, which has been stock culture in this laboratory for 12 years.

In order to avoid misunderstanding, it should be stated that in the first seven preventive, and the first six curative experiments, the cultures in medium-sized tubes were grown on slant agar for 24 hours in the incubator. Of these, $\frac{1}{8}$ of a culture was fatal to 250-gram guinea-pigs in 26 to 33 hours. Beginning with experiment 8 preventive, and with experiment 7 curative, the cultures were grown in *large* uniform test-tubes on slant agar. The fatal dose, thereupon, became $1/25$ culture.

Preventive Experiments.

Following the technique of Cruveilhier, a serum¹ of 43 units per c.c. was compared with an antitoxic solution globulin² of 1,700 units per c.c.

Four guinea-pigs were injected subcutaneously with $1/150,000$, $1/200,000$, $1/250,000$, $1/300,000$ of a c.c. per gram weight, of the 43-unit serum, respectively. A parallel set of four guinea-pigs received the same volume per gram weight of the 1,700 unit antitoxic globulin solution; 24 hours later each animal in both series, as well as a control guinea-pig, received subcutaneously a fatal dose of culture No. 8. The control animal died in $24\frac{1}{2}$ hours. The animals which received the 43-unit serum died in 25–28 hours, while those which received the antitoxic globulin solution remained normal. In the above experiment, the

¹ Obtained from Horse 308. This animal had received increasing amounts of toxin since March 6, 1906. The most potent antitoxic value was 250 units on May 3, 1906. Although receiving increasing amounts of toxin every day, the potency dropped steadily, and on July 7, 1906, seven days after the last injection of toxin (1,000 c.c. with a M. L. D. of 0.003), the antitoxic value was about 50 units. At the time of this experiment the serum tested 43 units per c.c.

² Prepared from 23 liters of citrated plasma (potency 700 units per c.c.) obtained from four bleedings of Horse 1.

1/150,000 c.c. per gram weight of the 43-unit serum contained 1/25 of a unit; while the same volume of antitoxic globulin solution contained $2\frac{3}{4}$ units.

In the second experiment, on account of the great difference in antitoxic unit content, the dilutions were made according to the number of units present.

Four guinea-pigs received 1/6,250, 1/12,500, 1/25,000 and 1/50,000 c.c. of the 43-unit serum, per gram weight, respectively. Two guinea-pigs received 1/425,000 and 1/850,000 c.c. per gram weight, respectively, of the 1,700-unit antitoxic globulin solution. Twenty-four hours later, all the animals, as well as a control, received a fatal dose of culture No. 8. Control animal died in 33–36 hours. The animal which received the 1/50,000 c.c. of the 43-unit serum showed marked induration and lost one-third of its weight. The two which received the 1/425,000 and 1/850,000 c.c. per gram weight of the antitoxic globulin solution remained normal.

In the third experiment made in conjunction with the second, a serum¹ of 200 units was compared with one² of 1,000 units.

Three guinea-pigs received 1/50,000, 1/100,000 and 1/200,000 c.c. of the 200-unit serum, per gram weight, respectively. Three others received 1/250,000, 1/500,000 and 1/1,000,000 c.c. of the 1,000-unit serum, per gram weight, respectively. Twenty-four hours later all the animals, as well as a control, received a fatal dose of culture No. 8. Control animal died in 33–36 hours. The animal which received 1/200,000 c.c. of the 200-unit serum and the one receiving 1/1,000,000 of the 1,000-unit serum showed marked induration and great loss of weight.

¹ Obtained from Horse 307. This animal received increasing amounts of toxin since February 14, 1906. The most potent antitoxic value was 350 units on May 3, 1906. Two months later, on July 5, 1906, seven days after the last injection of toxin (850 c.c. with an M. L. D. of 0.003), the antitoxic value was 225 units. At the time of this experiment the serum tested 200 units per c.c.

² Obtained from Horse 305. This animal had received increasing amounts of toxin since February 14, 1906. The most potent antitoxic value was 1,250 units on April 17, 1906. On June 12, 1906, seven days after last injection of toxin (500 c.c. with an M. L. D. of 0.002) the antitoxic value was 1,100 units. At the time of this experiment the serum tested 1,000 units per c.c.

In the fourth experiment, four guinea-pigs received $1/25,000$, $1/37,500$, $1/50,000$ and $1/75,000$ c.c. of the 43-unit serum, per gram weight, respectively. Four others received $1/1,000,000$, $1/1,500,000$, $1/2,000,000$, and $1/3,000,000$ c.c. of the 1,700-unit antitoxic globulin solution, per gram weight, respectively. Twenty-four hours later all the animals as well as a control received a fatal dose of culture No. 8. Control died in 28 hours. The animal which received the $1/37,500$ c.c. of the 43-unit serum was protected; the $1/50,000$ c.c. failed to protect. The animal which received the $1/1,500,000$ c.c. of the 1,700-unit antitoxic globulin solution was protected; the $1/2,000,000$ c.c. failed to protect.

In the fifth experiment, four guinea-pigs received $1/100,000$, $1/150,000$, $1/200,000$ and $1/250,000$ c.c. of the 200-unit serum, per gram weight, respectively. Four others received $1/500,000$, $1/750,000$, $1/1,000,000$ and $1/1,250,000$ c.c. of the 1,000-unit serum, per gram weight, respectively. Twenty-four hours later all the animals, as well as a control, received a fatal dose of culture No. 8. Control animal died in 28 hours. The animal which received the $1/150,000$ c.c. of the 200-unit serum was protected; the $1/200,000$ c.c. failed to protect. The animal which received the $1/750,000$ c.c. of the 1,000-unit serum was protected; the $1/1,000,000$ c.c. failed to protect.

Thus far our results were not in accord with those of Cruveilhier. In our experiments the therapeutic value of the serum appeared to be measured by the antitoxic unit content, rather than, as his experiments indicated, by the quantity of serum.

TABLE I.
Preventive Experiments 1 to 5.

Experiment No. 1 with Culture 8.				Experiment No. 3 with Culture No. 8.			
Fraction of c.c. per Gram Weight.	Serum of Horse 308 43 Units per c.c.	Fraction of c.c. per gram Weight.	Antitoxic Glob. Sol. 1,700 Units per c.c.	Fraction of c.c. per Gram Weight.	Serum of Horse 307 200 Units per c.c.	Fraction of c.c. per Gram. Weight.	Serum of Horse 305 1,000 Units per c.c.
1/150,000	died	1/150,000	lived	1/50,000	lived	1/250,000	lived
1/200,000	died	1/200,000	lived	1/100,000	lived	1/500,000	lived
1/250,000	died	1/250,000	lived	1/200,000	lived	1/1,000,000	lived
1/300,000	died	1/300,000	lived				
Control died in 24½ hours.				Control died in 33-36 hrs.			
Experiment No. 2 with Culture No. 8.				Experiment No. 5 with Culture No. 8			
1/6,250	lived	1/425,000	lived	1/100,000	lived	1/500,000	lived
1/12,500	lived	1/850,000	lived	1/150,000	lived	1/750,000	lived
1/25,000	lived			1/200,000	died	1/1,000,000	died
1/50,000	lived			1/250,000	died	1/1,250,000	died
Control died in 33-36 hrs.				Control died in 28 hrs.			
Experiment No. 4 with Culture No. 8.							
1/25,000	lived	1/1,000,000	lived				
1/37,500	lived	1/1,500,000	lived				
1/50,000	died	1/2,000,000	died				
1/75,000	died	1/3,000,000	died				
Control died in 28 hrs.							

In experiment 6, the 43-unit serum was compared with an antitoxic globulin solution¹ of 1,450 units. Four guinea-pigs received $\frac{3}{4}$, $\frac{1}{2}$, $\frac{1}{4}$ and $\frac{1}{8}$ unit of the 43-unit serum, respectively. A parallel set of four guinea-pigs received the same fractions of a unit of the 1,450-unit antitoxic globulin solution. Twenty-four hours later all the animals as well as a control received a fatal dose of culture No. 1. Control animal died in 26 hours. The $\frac{3}{4}$ unit of both the serum and the antitoxic

¹ Prepared from 22 liters of citrated plasma (potency 600 units per c.c.) obtained from two bleedings each of Horses J and L.

globulin solution protected, while all the animals which received the lower fractions died.

Experiment 7 was identical with the preceding, except that culture No. 2 was used in place of culture No. 1. The results were: Control animal died in 25 hours. The animal which received the $\frac{1}{2}$ unit of the 43-serum became greatly emaciated and barely survived. The $\frac{3}{4}$ unit of the same serum failed to protect. In all probability this difference was due to an idiosyncrasy of one of the animals. The $\frac{3}{4}$ unit of the 1,450-unit antitoxic globulin solution protected.

Experiments 6 and 7 were repeated in experiments 8 and 9. The antitoxic values injected were 1, $\frac{3}{4}$, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$ and $\frac{1}{16}$ unit. Using culture No. 1 for experiment No. 8, the results were: Control animal died in 26 hours. The $\frac{3}{4}$ unit of both the serum and antitoxin globulin solution protected.

In experiment 9, using culture No. 2, in place of culture No. 1, otherwise parallel with experiment 8, the results were: The control animal died in $23\frac{1}{2}$ hours. The one unit of both the serum and the antitoxic globulin solution protected.

In experiment 10, using culture No. 1, a serum¹ of 600 units was compared with one² of 1,300 units. The same antitoxic values were administered as in experiments 8 and 9. The results were: Control animal died in 23 hours. The one unit of both sera protected.

In experiment 11, using culture No. 2, in place of culture No. 1, otherwise parallel with experiment 10, the results were: Control animal died in 33–36 hours. The $\frac{1}{4}$ unit of both sera barely protected.

In experiment 12, using culture No. 8, in place of No. 2, otherwise parallel with experiments 10 and 11, the results were: Control animal

¹Obtained from Horse N. This animal had received increasing amounts of toxin since October 5, 1906. The most potent antitoxic value was 700 units on February 26, 1907, seven days after the last injection of toxin. Rebleeding one week later, the potency had dropped to 600 units. This was its value also at the time of our experiments, two weeks later.

²Obtained from Horse 306. This animal had received increasing amounts of toxin since February 14, 1906. The most potent antitoxic value was 1,450 units on May 18, 1906, six days after last injection of toxin (550 c.c. with an M. L. D. of 0.002). At the time of these experiments the serum tested 1,300 units per c.c.

died in 28 hours. The $\frac{3}{4}$ unit of the 600-unit serum protected; the $\frac{1}{2}$ unit of the 1,300-unit serum barely protected.

In the two following experiments (13 and 14), comparisons were made of the three sera¹ of different unit content obtained from the same horse during the course of immunization.

In experiment 13, using culture No. 1, five guinea-pigs received 1, $\frac{3}{4}$, $\frac{1}{2}$, $\frac{1}{4}$ and $\frac{1}{8}$ unit of a 600-unit serum respectively, five others received the same antitoxic unit values of a 335-unit serum; five more received the same values of a 200-unit serum. Twenty-four hours later all the animals, as well as a control, received a fatal dose of culture No. 1. The results were: Control animal died in 28 hours. The $\frac{1}{2}$ unit of each of the three sera protected.

In experiment 14, using culture No. 8 in place of culture No. 1, otherwise parallel with experiment No. 13, the results were: Control animal died in 30 hours. The $\frac{1}{2}$ unit of each of the three sera protected.

To summarize: In experiments 1, 2, 4, 6, 7, 8 and 9, we compared a 43-unit native serum with a 1,700-unit and a 1,450-unit antitoxin globulin solution. The results, in each case, are seen to depend on the antitoxic unit content of the serum and not on its volume. The same is true of experiments 3 and 5, in which a 200-unit serum was compared with one of 1,000 units, and of experiments 10, 11 and 12, in which a fresh serum (two weeks old), of 600 units was compared with one (10 months old), of 1,300 units. Finally the same results are seen in experiments 13 and 14, in which we compared three bleedings from the same horse as follows: One bleeding originally contained 625 units per c.c. (this was the maximum value attained by the animal). The second contained 335 units per c.c., and the third contained 200 units per c.c.

¹ Obtained from Horse 322. This animal had received increasing amounts of toxin since March 7, 1907. The most potent antitoxic value was 625 units, on April 30, 1907. Tested at the time of these experiments the serum contained 600 units per c.c. After the fourth bleeding, which was on July 7, 1907, the antitoxic value had dropped to 340 units; tested at the time of these experiments the serum contained 335 units per c.c. After three further bleedings, the antitoxic value dropped to 200 units on June 28, 1907. This was the potency also at the time of these experiments.

TABLE 2.
Preventive Experiments 6 to 14.

Amount Injected in Units.	Experiment No. 6 with Culture No. 1		Experiment No. 10 with Culture No. 1		Experiment No. 13 with Culture No. 1		
	Serum of Horse 308 43 Units per c.c.	Antitoxic Glob. Sol. 1,415 Units per c.c.	Serum of Horse N 600 Units per c.c.	Serum of Horse 306 1,300 Units per c.c.	Serum of Horse 322 600 Units per c.c.	Serum of Horse 322 335 Units per c.c.	Serum of Horse 322 200 Units per c.c.
1 unit			lived	lived	lived	lived	lived
$\frac{3}{4}$ "	lived	lived	died	died	lived	lived	lived
$\frac{1}{2}$ "	died	died	died	died	lived	lived	lived
$\frac{1}{4}$ "	died	died	died	died	died	died	died
$\frac{1}{8}$ "	died	died	died	died	died	died	died
$\frac{1}{16}$ "			died	died			
	Control died in 26 hrs.		Control died in 23 hrs.		Control died in 28 hrs.		
	Experiment No. 7 with Culture No. 2		Experiment No. 11 with Culture No. 2		Experiment No. 14 with Culture No. 8		
1 unit			lived	lived	lived	lived	lived
$\frac{3}{4}$ "	died	lived	lived	lived	lived	lived	lived
$\frac{1}{2}$ "	lived	died	lived	lived	lived	lived	lived
$\frac{1}{4}$ "	died	died	lived	lived	died	died	died
$\frac{1}{8}$ "	died	died	died	died	died	died	died
$\frac{1}{16}$ "			died	died			
	Control died in 25 hrs.		Control died in 33-36 hrs.		Control died in 30 hrs.		
	Experiment No. 8 with Culture No. 1		Experiment No. 12 with Culture No. 8				
1 unit	lived	lived	lived	lived			
$\frac{3}{4}$ "	lived	lived	lived	lived			
$\frac{1}{2}$ "	died	died	died	lived			
$\frac{1}{4}$ "	died	died	died	died			
$\frac{1}{8}$ "	died	died	died	died			
$\frac{1}{16}$ "	died	died	died	died			
	Control died in 26 hrs.		Control died in 28 hrs.				
	Experiment No. 9 with Culture No. 2						
1 unit	lived	lived					
$\frac{3}{4}$ "	died	died					
$\frac{1}{2}$ "	died	died					
$\frac{1}{4}$ "	died	died					
$\frac{1}{8}$ "	died	died					
$\frac{1}{16}$ "	died	died					
	Control died in 23½ hrs.						

Curative Experiments.

Experiment 1—Following Cruveilhier's technique, we infected the animals with culture No. 8, and divided them into two lots. At intervals of two hours, from the second to the tenth hour, each animal of the first lot received subcutaneously 25 units of the 43-unit serum; and each animal of the second lot, 25 units of the 1,700-unit antitoxic globulin solution. The results of both lots were uniform. Control animal died in 25 hours. The animals which received the curative dose after 2 and 4 hours lived; while those receiving the curative dose after 6, 8 and 10 hours, died.

Experiment 2—The animals were infected with culture No. 1, and divided into two lots. At intervals of two hours, from the second to the eighth hour, each animal of the first lot received subcutaneously 25 units of the 43-unit serum; and each animal of the second lot, 25 units of the 1,450-unit antitoxic globulin solution. The results of both lots were uniform. Control animal died in 26 hours. The animals which received the curative dose after 2, 4 and 6 hours lived; while those receiving the curative dose after 8 hours, died.

Experiment 3—The animals were infected with culture No. 2, in place of culture No. 1; otherwise parallel with experiment 2. The results of both lots were again uniform. The control animal died in 26 hours. The animals which received the curative doses after 2 and 4 hours lived; after 6 and 8 hours, died.

Experiment 4—Repeating experiment 2, in duplicate, the result of both lots were again uniform. The control animal died in 21½ hours. The animals which received the curative dose after 2 and 4 hours lived; after 6 and 8 hours, died.

Experiment 5—Repeating experiment 3, in duplicate, the results were again uniform. The control animal died in 28 hours. The animals which received the curative dose after 2, 4 and 6 hours, lived; after 8 hours, died.

Experiment 6—The animals were injected with culture No. 1, divided in two lots. At intervals of two hours, from the fourth to eighth hour, each animal of the first lot received subcutaneously units of the 600-unit serum. The second lot received 25 units of

1,300-unit serum. The results of both lots were uniform. The control animal died in 27 hours. The animals which received the curative dose after 4 and 6 hours, lived; after 8 hours, died.

Experiment 7—The animals were infected with culture No. 2 in place of culture No. 1, otherwise parallel with experiment No. 6. The results of both lots were uniform. The control animal died in 28 hours. The animals which received the curative dose after 4 and 6 hours lived; after 8 hours, died.

TABLE 3.
Curative Experiments 1 to 11.

Hours Elapsed after Injection of Culture.	Experiment 1 with Culture 8.		Experiment 2 with Culture 1.		Experiment 6 with Culture 1.		Experiment 9 with Culture 1.		
	Serum of Horse 308 43 Units per c.c. (25 Units Injected).	Antitoxic Glob. Sol. 1,700 Units per c.c. (25 Units Injected).	Serum of Horse 308 43 Units per c.c. (25 Units Injected).	Antitoxic Glob. Sol. 1,450 Units per c.c. (25 Units Injected).	Serum of Horse N 600 Units per c.c. (25 Units Injected).	Serum of Horse 306 1,300 Units per c.c. (25 Units Injected).	Serum of Horse 122 600 Units per c.c. (25 Units Injected).	Serum of Horse 122 335 Units per c.c. (25 Units Injected).	Serum of Horse 122 200 Units per c.c. (25 Units Injected).
2 hours	lived	lived	lived	lived	lived	lived	lived	lived	lived
4 "	lived	lived	lived	lived	lived	lived	lived	lived	lived
6 "	died	died	lived	lived	lived	lived	died	died	died
8 "	died	died	died	died	died	died	died	died	died
	Control died in 25 hrs.		Control died in 26 hrs.		Control died in 27 hrs.		Control died in 22 hrs.		
	Experiment 1 with Culture 8.		Experiment 3 with Culture 2.		Experiment 7 with Culture 2.		Experiment 10 with Culture 8.		
	Serum of Horse 308 43 Units per c.c. (25 Units Injected).	Antitoxic Glob. Sol. 1,700 Units per c.c. (25 Units Injected).	lived	lived	lived	lived	lived	lived	lived
	lived	lived	lived	lived	lived	lived	died	died	died
	died	died	died	died	died	died	died	died	died
	Control died in 25 hrs.		Control died in 26 hrs.		Control died in 26 hrs.		Control died in 24½ hrs.		

Experiment 8—The animals were infected with culture No. 8, in place of culture No. 2, otherwise parallel with experiments 6 and 7. The results of both lots were uniform. The control died in 27 hours. The animals which received the curative dose after 4 and 6 hours lived; after 8 hours, died.

Experiment 9—The animals were infected with culture No. 1 and divided into three lots. At intervals of two hours, from the fourth to the eighth hour, each animal of the first lot received subcutaneously 25 units of the 600-unit serum, the second lot, 25 units of the 335-unit serum, and the third lot received the same number of units of the 200-unit serum. These three sera were obtained from the same horse during the course of immunization. The results of the three lots were uniform. The control died in 22 hours. The animals which received the curative dose after 4 hours lived; after 6 and 8 hours, died.

Experiment 10—The animals were infected with culture No. 8 in place of culture No. 1, otherwise parallel with experiment 9. The results of the three lots were uniform. The control animal died in 24½ hours. Those which received the curative dose after 4 hours lived; after 6 and 8 hours, died.

Experiment 11—The animals were infected with culture No. 2, in place of culture No. 1; otherwise parallel with experiments 9 and 10. The results of the three lots were uniform. The control died in 27 hours. Those which received the curative dose after 4 and 6 hours lived; after 8 hours, died.

Conclusions.

In view of the results obtained in the comparison of the antitoxic content of sera, both preventively and curatively, it is obvious that in diphtheria in the guinea-pig, the therapeutic value of antidiphtheric sera depends on the number of antitoxic units present. There is no reason for believing that conditions in man are different.

According to our experiments antidiphtheric serum contains no protective substances, aside from the antitoxin, which play an important rôle therapeutically.

The present method of standardizing antidiphtheric serum accurately measures its therapeutic value.

THE RELATIVE THERAPEUTIC VALUE OF ANTITOXIC GLOBULIN SOLUTION AND THE WHOLE SERUM FROM WHICH IT WAS DERIVED.

DRS. EDNA STEINHARDT and EDWIN J. BANZHAF.

The process of concentrating diphtheria antitoxin, devised in this laboratory by Gibson,¹ has been adopted by a large number of American manufacturers, and has resulted in the extensive employment of the product in this country. Careful clinical trials have convinced us that this antitoxic globulin preparation possesses all the therapeutic properties of the native serum.

Cruveilhier² has recently stated that the customary method of standardizing antidiphtheric serum is not comprehensive enough, in that it leaves out of account therapeutic serum-constituents other than the antitoxin. While we believe that the work of Steinhardt and Banzhaf in the preceding paper³ has effectually disposed of this objection, it seemed advisable to study the question in connection with the Gibson process of concentration, and with the fractional precipitation of the antiserum according to the method of Banzhaf and Gibson.⁴

Our general method of procedure was similar to that outlined in the preceding paper. In this instance, we mixed antitoxic citrated plasma from several horses and then tested this mixture before and after eliminating the non-antitoxic proteins. We also compared the different globulin fractions of the plasma.

In these experiments, the following mixtures of citrated plasma were used:

Mixture 1—Fifty liters of citrated plasma were obtained from five horses as follows:

1,000 c.c.	from horse 262,	part of one bleeding.
3,600 c.c.	" "	305, " " two bleedings.
5,400 c.c.	" "	306, " " three bleedings.
1,500 c.c.	" "	310, " " one bleeding.
38,500 c.c.	" "	311, full six bleedings.

The potency of this mixture, at the time of these experiments, was 700 units per c.c.

¹ *Jour. Biol. Chem.*, 1, p. 161.

² *Ann. de l'Inst. Past.*, 1904, 18, p. 249.

³ *Jour. Infect. Dis.*, 1908, 5, p. 203.

⁴ *Jour. Biol. Chem.*, 3, p. 253.

Mixture 2—Forty liters of citrated plasma were obtained from three horses as follows:

8,000 c.c.	from horse 288,	full bleeding.
6,100 c.c.	“ “	305, full bleeding.
25,000 c.c.	“ “	299, full four bleedings.

The original potency of this mixture was 475 units per c.c. At the time of these experiments, however, the potency had dropped to 450 units per c.c.

(Mixture 1.) The 50 liters were refined and concentrated by Gibson's method, to 16 liters of antitoxic globulin solution containing 1,650 units per c.c.

Gibson's method briefly is as follows: The diluted citrated plasma is precipitated with an equal amount of saturated ammonium sulphate solution and the antitoxic proteins are separated by extracting the precipitate with saturated sodium chloride solution. The soluble antitoxic proteins are then reprecipitated from the saturated sodium chloride solution with acetic acid. This filtered precipitate is then partially dried between filter papers and dialyzed in parchment in running water.

(Mixture 2.) The 40 liters were divided into two lots. One lot of 20 liters was diluted with an equal amount of water and refined and concentrated by Gibson's method. The final product, designated 77A, amounted to 5,200 c.c., each c.c. containing 1,450 units per c.c. At the time of these experiments, however, the potency had dropped to 1,375 units per c.c.

The second lot of 20 liters was diluted with an equal amount of water and the antitoxic globulins obtained by three fractional precipitations at concentrations corresponding to 3.3 c.c., 3.3–3.8 c.c. and 3.8–5.0 c.c. of saturated ammonium sulphate in 10 c.c.¹

¹ There exists at the present time considerable confusion in comprehending the methods and basic principles of ammonium sulphate fractional precipitation of proteins. The nomenclature which we have employed and which designates the number of c.c. of saturated ammonium sulphate solution in 10 c.c. of the *precipitated* mixture has been used by some authorities; it avoids the confusion developed by the use of such terms as “per cent $(\text{NH}_4)_2\text{SO}_4$ solution,” per cent saturation $(\text{NH}_4)_2\text{SO}_4$,” “per cent of saturated $(\text{NH}_4)_2\text{SO}_4$ solution,” and “per cent saturation $(\text{NH}_4)_2\text{SO}_4$ solution,” and it seems the simplest and best *practical* expression of degrees of saturation yet suggested. We advise that this method be employed in future papers on fractional precipitation (see page 98).

The soluble antitoxic proteins in saturated sodium chloride solution were prepared in the usual way. The antitoxic globulin solution from the first fraction, designated 77B, amounted to 1,440 c.c., each c.c. containing 1,150 units per c.c. At the time of these experiments, however, the potency had dropped to 1,025 units per c.c. The second fraction, designated 77C, amounted to 1,400 c.c., each c.c. containing 1,350 units per c.c. At the time of these experiments, however, its potency had dropped to 1,175 units per c.c. The third fraction, designated 77D, amounted to 2,050 c.c., each c.c. containing 1,750 units per c.c. At the time of these experiments, however, the potency had dropped to 1,550 units per c.c.

The animals used in these experiments were active, healthy, normal guinea-pigs weighing between 250–260 grams.

The three diphtheria strains were the same as in the preceding paper.

PREVENTIVE EXPERIMENTS.

Mixture 1.

A uniform sample of the mixture of citrated plasma containing 700 units per c.c., was compared with the finished product of antitoxic globulin solution containing 1,650 units per c.c.

Experiment 1—Six guinea-pigs were inoculated subcutaneously with 1, $\frac{3}{4}$, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$ and $\frac{1}{16}$ -unit of the 700-unit citrated plasma, respectively; a parallel set of six guinea-pigs received the same antitoxic unit values of the 1,650 antitoxic globulin solution. Twenty-four hours later all the animals, as well as a control guinea-pig, received a fatal dose of culture No. 1.¹ The control animal died in 27 hours. The $\frac{3}{4}$ -unit of both the citrated plasma and the antitoxic globulin solution protected.

Experiment 2—In this, culture No. 2¹ was used in place of culture No. 1; otherwise it was parallel with the first experiment. The results were: The control animal died between 33 and 36 hours. The $\frac{1}{4}$ -unit of both the citrated plasma and the antitoxic globulin solution protected.

¹ Culture Nos. 1 and 2: No. 1, a moderate toxin producer; No. 2, a weak toxin producer. Both of these were freshly isolated from the throats of diphtheria patients at the Department of Health Hospital.

Experiment 3—In this, culture No. 8¹ was used in place of culture No. 2; otherwise it was parallel with the first and second experiments. The results were: The control animal died in 28 hours. The ½-unit of both the citrated plasma and the antitoxic globulin solution protected.

TABLE I.
Preventive Experiments.

Amount Injected in Units.	Experiment 1 with Culture No. 1.		Experiment 4 with Culture No. 1.				
	Sample of Plasma 700 Units per c.c.	Concentrated Anti-toxic Glob. Sol. 1,650 Units per c.c.	Sample of Plasma 450 Units per c.c.	Concentrated Anti-toxic Glob. Sol. 77A 1,375 Units per c.c.	First Fract. Antitoxic Glob. Sol. 77B 1,025 Units per c.c.	Second Fract. Anti-toxic Glob. Sol. 77C 1,175 Units per c.c.	Third Fract. Anti-toxic Glob. Sol. 77D 1,550 Units per c.c.
1 unit	lived	lived	lived	lived	lived	lived	lived
¾ "	lived	lived	lived	lived	lived	lived	lived
½ "	died	died	lived	lived	lived	lived	lived
¼ "	died	died	died	died	died	died	died
⅛ "	died	died	died	died	died	died	died
⅙ "	died	died	died	died	died	died	died
Control died in 27 hrs.			Controls died in 28½ and 29 hrs.				
	Experiment 2 with Culture No. 2.		Experiment 5 with Culture No. 2.				
1 unit	lived	lived	lived	lived	lived	lived	lived
¾ "	lived	lived	lived	lived	lived	lived	lived
½ "	lived	lived	lived	lived	lived	lived	lived
¼ "	lived	lived	died	died	died	died	died
⅛ "	died	died	died	died	died	died	died
⅙ "	died	died	died	died	died	died	died
Control died in 33-36 hrs.			Controls died in 28½ hrs.				
	Experiment 3 with Culture No. 8.		Experiment 6 with Culture No. 8.				
1 unit	lived	lived	lived	lived	lived	lived	lived
¾ "	lived	lived	lived	lived	lived	lived	lived
½ "	lived	lived	lived	lived	lived	lived	lived
¼ "	died	died	lived	lived	lived	lived	lived
⅛ "	died	died	died	died	died	died	died
⅙ "	died	died	died	died	died	died	died
Control died in 28 hrs.			Controls died in 30-32 hrs.				

¹ Park and Williams, Culture No. 8. This is a strong toxin producer and has been the stock in this laboratory for 12 years.

Mixture 2.

In Experiments 4, 5 and 6, we instituted the following comparisons:

a) Between a sample of citrated plasma (Mixture 2) containing 450 units per c.c., and the finished product of antitoxic globulin solution containing 1,375 units per c.c.

b) Between the same citrated plasma (Mixture 2), and the three fractions of the antitoxic globulins; of these Fraction 1 contained 1,025 units per c.c.; Fraction 2 contained 1,175 units per c.c.; and Fraction 3, 1,550 units per c.c.

Experiment 4—This required 30 guinea-pigs as follows: Six guinea-pigs received 1, $\frac{3}{4}$, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$ and $\frac{1}{16}$ -unit of the 450-unit citrated plasma, respectively; six received the same antitoxic unit values of the 1,375-unit antitoxic globulin solution, 77A; six received the same antitoxic values of the 1,025-unit, first fraction, antitoxic globulin solution; six received the same antitoxic values of the 1,175-unit, second fraction, antitoxic globulin solution; six received the same values of the 1,550-unit, third fraction, antitoxic globulin solution. Twenty-four hours later, all the animals, as well as two control guinea-pigs, received a fatal dose of culture No. 1. The results of the five lots were uniform. The control animals died between $28\frac{1}{2}$ and 29 hours. The $\frac{1}{2}$ -unit of the citrated plasma, the antitoxic globulin solution, and the fractions of the antitoxic globulins protected.

Experiment 5—In this, culture No. 2 was used in place of culture No. 1; otherwise it was parallel with experiment 4. The results of the five lots were uniform. The control animals died in $28\frac{1}{2}$ hours. The $\frac{1}{2}$ -unit of the citrated plasma, the antitoxic globulin solution, and the fractions of the antitoxic globulins protected.

Experiment 6—In this, culture No. 8 was used in place of culture No. 2; otherwise it was parallel with experiments 4 and 5. The results of the five lots were uniform. The control animals died between 30 and 32 hours. The $\frac{1}{4}$ -unit of the citrated plasma, the antitoxic globulin solution, and the fractions of the antitoxic globulins protected.

CURATIVE EXPERIMENTS.

Mixture 1.

The guinea-pigs were inoculated subcutaneously with a fatal dose of culture No. 1, and divided into two lots. At intervals of two hours from the fourth to the eighth hour, each animal of the first lot received subcutaneously 25 units of the 700-unit citrated plasma and each animal of the second lot received 25 units of the 1,650-unit antitoxic globulin solution. The results were: The control animal died in 27 hours. The animals receiving the curative dose after four and six hours, lived; while those receiving the curative dose after eight hours, died.

TABLE 2.
Curative Experiments.

No. of Hours after Injection of Culture	Experiment 1 with Culture No. 1		Experiment 4 with Culture No. 1				
	Sample of Plasma 700 Units per c.c. (25 Units Injected)	Concen- trated Antitoxic Glob. Sol. 1,650 Units per c.c. (25 Units Injected)	Sample of Plasma 450 Units per c.c. (25 Units Injected)	Concen- trated Anti- toxic Glob. Sol. 77A 1,375 Units per c.c. (25 Units Injected)	First Fract. Antitoxic Glob. Sol. 77B 1,025 Units per c.c. (25 Units Injected)	Second Fract. Anti- toxic Glob. Sol. 77C 1,175 Units per c.c. (25 Units Injected)	Third Fract. Anti- toxic Glob. Sol. 77D 1,550 Units per c.c. (25 Units Injected)
4 hours	lived	lived	lived	lived	lived	lived	lived
6 "	lived	lived	lived	lived	lived	lived	lived
8 "	died	died	died	died	died	died	died
	Control died in 27 hrs.		Controls died in 28 hrs.				
	Experiment 2 with Culture No. 2		Experiment 5 with Culture No. 2				
	lived	lived	lived	lived	lived	lived	lived
4 hours	lived	lived	lived	lived	lived	lived	lived
6 "	lived	lived	lived	lived	lived	lived	lived
8 "	died	died	died	died	died	died	died
	Control died in 26½ hrs.		Controls died in 27-27½ hrs.				
	Experiment 3 with Culture No. 8		Experiment 6 with Culture No. 8 (in Duplicate)				
	lived	lived	2 lived	2 lived	2 lived	2 lived	2 lived
4 hours	lived	lived	2 lived	2 lived	2 lived	2 lived	2 lived
6 "	died	died	2 died	2 died	2 died	2 died	2 died
8 "	died	died	2 died	2 died	2 died	2 died	2 died
	Control died in 25½ hrs.		Controls died in 25 hrs.				

Experiment 2—In this, culture No. 2 was used in place of culture No. 1; otherwise it was parallel with the first experiment. The results were: The control animal died in 26½ hours. The animals receiving the curative dose after four and six hours, lived; while those receiving the curative dose after eight hours, died.

Experiment 3—In this, culture No. 8 used in place of culture No. 2; otherwise it was parallel with experiments 1 and 2. The results were: The control animal died in 25½ hours. The animals receiving the curative dose after four hours, lived; after six and eight hours, died.

Mixture 2.

Experiment 4—The guinea-pigs received subcutaneously a fatal dose of culture No. 1, and were divided into five lots. At intervals of two hours from the fourth to the eighth hour, each animal of the first lot received subcutaneously 25 units of the 450-unit citrated plasma; each animal of the second lot 25 units of the 1,375-unit antitoxic globulin solution, 77A; the third lot 25 units of the 1,025-unit, first fraction, antitoxic globulin solution; the fourth lot 25 units of the 1,175-unit, second fraction, antitoxic globulin solution; the fifth lot 25 units of the 1,550-unit, third fraction, antitoxic globulin solution. The results of the five lots were uniform. The control animals died in 28 hours. All the animals receiving the curative dose after four and six hours, lived; after eight hours, died.

Experiment 5—In this, culture No. 2 was used in place of culture No. 1; otherwise it was parallel with experiment 4. The results of the five lots were again uniform. The control animals died in 27 and 27½ hours. All the animals receiving the curative dose after four and six hours, lived; after eight hours, died.

Experiment 6—In this, which was in duplicate, culture No. 8 was used, in place of culture No. 2; otherwise it was parallel with experiments 4 and 5. The duplicate results of the five lots were again uniform. The control animals died in 25 hours. All the animals receiving the curative dose after four hours, lived; after six and eight hours, died.

In view of the results obtained in these experiments and in those of Steinhardt and Banzhaf in the preceding paper, it is obvious that the therapeutic value of the plasma is not appreciably impaired through the process of eliminating the albumins and other non-antitoxic proteins by the salting out methods employed, and the final dialyzation of the concentrated product.

A NOTE ON ANAPHYLAXIS.

By Drs. EDWIN J. BANZHAF and L. W. FAMULENER.

Rosenau and Anderson in their first communication on hypersusceptibility reported their attempts to destroy or remove the toxic substances from horse serum. They treated the serum, which was used for the second injection, by various physical and chemical agents, such as heating the serum to 60 degrees C., filtering through porcelain, drying, freezing, precipitation and dialysis, and by the direct addition to the serum for various lengths of time of such chemicals as potassium permanganate, hydrogen peroxide, succinic acid and butyric acid, also various antiseptics, all without success. Continuing their investigations they reported, in their second communication, the influence of ferments, alkaloids, salts and such substances as ox bile, animal charcoal and yeast cells. These also gave negative results.

One of us took up the problem from another standpoint, *i. e.*, to treat the sensitized animal with drugs just prior to the second injection. In the preliminary experiments morphine sulphate was used with negative results. Following this, chloral hydrate was employed to produce hypnosis, and it was found that sensitized guinea pigs could be protected with this drug. After our experiments with chloral hydrate were well advanced, Besredka's communication came to our attention, in which he stated that ether and also calcium chloride exerted a protective action when administered to sensitized guinea-pigs before the second injection of the serum.

We found by injecting a solution of chloral hydrate which was just sufficient to produce hypnosis, that fully 75 per cent. of all serum sensitized guinea pigs were completely protected from the second injection of serum into the peritoneal cavity, while 90 per cent. of all the controls died. We believe that with improved technique in the dosage of chloral hydrate it is possible to protect 90 per cent. of all fully sensitized guinea-pigs. By "fully sensitized," we mean that three weeks or a month should elapse before the second injection of serum into guinea-pigs which have survived the routine testing of antitoxin. With guinea-pigs which have received horse serum alone (1/100 to 1/500

c.c.) at least seven or eight weeks should elapse before the second injection. By allowing the above interval of time to elapse, over 90 per cent. of our controls died within an hour, most of them within 20 minutes.

We have found that the dose of the chloral hydrate per gram weight of the animal was more or less variable, no fixed amount can be stated, much depending upon the individual idiosyncrasy of the animal. Approximately 75 milligrams of the drug to a 250-gram guinea-pig, and 100 milligrams to a 300-gram guinea-pig produce the degree of hypnosis desired.

We use a fresh 10 per cent. solution of chloral hydrate, carefully measuring out the required amount into a small sterile beaker and adding an equal amount of sterile water. This diluted solution is injected into the muscles of the thigh of the animal, half into one leg and half into the other. If, after 20 to 30 minutes, the needle is inserted into the peritoneal cavity, muscular twitching and slight movement of the head will be noticed. The injection of 5 c.c. serum is then given and the animal kept in a warm room. No symptoms appear and the sleep is undisturbed. After $1\frac{1}{2}$ to $2\frac{1}{2}$ hours have elapsed, the animal slowly recovers from the effects of the drug. No symptoms or ill effects have been observed in any of the animals. Observations have been followed for over two weeks after treatment. The animal, after the effects of the drug have disappeared, will react with characteristic symptoms of anaphylaxis if given a third injection of serum. Up to the present time we have reinjected only 24 to 72 hours after recovery from the effects of the drug.

If the dose of chloral hydrate has not been sufficient, the insertion of the needle into the peritoneal cavity will cause pronounced muscular movements, raising of the head and an attempt to regain its feet. Under these conditions if the serum is injected the animal will die of anaphylaxis.

On the other hand, if the animal shows no muscular twitchings whatever, the dose of chloral has probably been too large. We wish to emphasize the fact that great care must be used not to overdose the sensitized guinea-pig with chloral hydrate, although a sensitized, as well as a normal guinea-pig, will recover from a large dose, consider-

ably more than the amount mentioned above. Apparently the combined effects of the drug and the serum in a sensitized animal produces a deeper hypnosis than the drug when given alone.

Thus far our experiments have been only with the intraperitoneal injection. Besredka's method of injecting directly into the brain will be taken up, and also the method of injecting directly into the vessels, according to Gay and Southard, and into the heart, according to Lewis.

In a number of experiments with calcium chloride, which, according to Besredka, exerts a protective action, we were unable to save the sensitized animals. They died with characteristic symptoms of anaphylaxis.

We have also failed to substantiate Besredka's claims that sensitized guinea-pigs under the influence of ether narcosis are protected from the second injection of $\frac{1}{4}$ c.c. of serum into the brain. Nor did we find that ether narcosis protected them when the injection was made directly into the vessel or directly into the heart. All the animals died with characteristic symptoms of anaphylaxis in from 2 to 6 minutes. Normal control guinea-pigs under the influence of ether narcosis showed no symptoms or ill effects when subjected to injections of physiological saline solution by the above methods.

In conclusion we wish to add that probably other chemical substances belonging to the same group as chloral will show similar action in protecting guinea-pigs against anaphylaxis.

A STUDY OF THE INTESTINAL FLORA.

KATHERINE R. COLLINS, M. D., and MARIE GRUND, M. D.

(Preliminary Report).

Although the literature shows that much detailed work on intestinal bacteria has been carried on in the last ten or fifteen years, still most of the investigators even within the past few years have worked severally along special lines, and but few attempts have been made to include the many branches of the subject in one work and thus present a comprehensive study of the bacterial flora of the intestinal tract in health and disease. While the main object of the present work is the study of the bacterial causes of summer diarrhoeas in infants, it is believed that the rational way is to precede this by an investigation of the intestinal flora of normal infants under different conditions of diet, etc., and by the study of the intestinal disturbances, complicating other maladies. From this brief outline of the field to be covered, it is perfectly apparent that the present studies which have been pursued for the past three months can not yet have progressed far enough to justify the publication of any data, and this preliminary report is intended merely to define in a general way, the scope of the work to be undertaken.

Considerable difficulty has been encountered in securing from plate cultures returns that seem proportionate to the number and variety of bacteria found in the stained films from fecal material, and some time has been spent in finding suitable media and in evolving a satisfactory technique. Material from infants was obtained chiefly from the obstetric and pediatric wards of the New York Infirmity for Women and Children, and from the dispensary of the same institution. To the time of writing 22 stools have been examined; of these 4 were meconium stools, and 4 were from children suffering from some variety of intestinal derangement. Of the 14 normal children whose stools were examined, 3 were under one month of age, 9 were between 1 and 12 months, and 2 were over 2 years old. Five children were breastfed exclusively, 5 had had breast feeding for some time, but had been fed on cows' milk at least two weeks before examination; 2, under 1 year, were on a milk mixture (malt soup), and 2 had received a mixed diet for some

time. Two of the meconium stools produced no growth in the media inoculated, while from one a fine growth of a Gram-positive coccus was obtained, but curiously enough the fourth yielded four bacterial varieties from plate cultures. This is the more striking since, with one exception, the number of varieties isolated from any one stool did not exceed three; in six cases, in fact, only one kind was obtained. Provided the meconium stools are excluded, our tests show that bacilli of the colon type were recovered from all but three of the cases examined.

In the stool of one very young breast-fed infant, smears and cultures showed only the Gram-positive coccus mentioned above, while in two cases the organism obtained was a bacillus which resembles the *B. coli* morphologically and culturally, except in its action on gelatin. This same bacillus was found associated with bacillus coli and other bacteria in three cases.

Several modifications of the ordinary nutrient agar were employed; besides the various carbohydrates (maltose, lactose, glucose, mannite, etc.), bile agar, beerwort-agar and whey-agar were used. The last-named has yielded rather better results than the others. The method of procedure in general has been the following: One gram of fecal material, obtained as fresh as possible, was rubbed up with 10 c.c. of sterile broth. One loopful of this emulsion was inoculated into a tube of cooled melted agar, and made into plates, which were then grown under anaerobic and aerobic conditions, at incubator temperature from one to three days.

So far, more time has been devoted to the study of infants' stools, but a few adult stools have been examined, and while these have shown a greater variety of organisms, the films made directly from fresh feces, as well as the returns from plate cultures, indicated considerable similarity in the flora of the two kinds of material. It would, of course, be premature to base conclusions on the small number of cases examined. Coccal forms are frequently found in both; a very large, slightly lance-shaped diplococcus, stained by Gram's method, which occurs almost constantly in the adult stools, has been found in about 33 per cent. of the infant cases; and a bacillus of the colon type, encountered in the majority of adult stools, was isolated from about 80 per cent. of the material from infants.

THE PERSISTENCE OF ANTHRAX AND TETANUS SPORES DURING THE PROCESS OF MAKING GELATIN.

KATHARINE R. COLLINS, M. D.

The following experiments were undertaken for the purpose of demonstrating the length of time that anthrax and tetanus spores may persist in infected tissues which have been subjected to the gelatin process:

A calf was infected with a large amount of anthrax spores and bacilli and was then killed. Pieces of the bone and skin were removed and placed in an incubator at 35 degrees C. for twenty-four hours. Examined the next day, these tissues were found to contain large numbers of anthrax spores. The pieces of bone and skin were thoroughly dried and placed in the solutions as below mentioned. The skin and bones of another calf were then prepared by soaking both pieces of bone and of skin in a thick emulsion of tetanus spores. The specimens were then dried and after testing were found to contain many living tetanus germs. These were also subjected to treatment below mentioned.

Tests with Anthrax Spores.

The skin from the infected calf was cut into strips about one-half inch wide, washed thoroughly and subjected to running water for eighteen hours. It was then placed into a lime bath for ten days, which was changed twice during this time. Taken from this bath it was thoroughly washed and all hair removed and then placed again into fresh lime water for four weeks.

At the end of two weeks tests were made and in the smears made directly from the material, well stained spores and bacilli were found. Cultures made from the same strips of skin gave a good growth of anthrax bacilli showing the spores were still alive. At the end of four weeks this same condition still existed. A portion of the skin was then heated in a water bath at 170 degrees F. for thirty minutes and cultures made from this were found to be sterile. Four weeks in the lime bath, together with heating to 170 degrees F. for thirty minutes, was sufficient to kill anthrax spores in the skin.

The bones from this same animal were treated as follows:

They were broken into small pieces, washed with water thoroughly and soaked in a bath of ten per cent. hydrochloric acid for three weeks, the bath being changed occasionally. At the end of three weeks well stained spores and bacilli were found in smears made from this gelatinous substance, and when cultures were made, the spores developed.

After thoroughly washing, the decalcified bone was placed in a lime bath for four weeks, the bath being changed occasionally. After being in the bath two weeks the spores were still active, but at the end of four weeks no growth occurred in the broth inoculated with the gelatinous material.

Some of this gelatinous material was heated, as in the case of the skin, and cultures made, which also showed no growth.

Tests upon bones and skin infected with tetanus spores:

Bones smeared with tetanus spores and bacilli were treated in the same way as the above. No living tetanus bacilli or spores remained alive after two weeks in the lime bath, as in case of the anthrax, although they were still active after being three weeks in the bath of hydrochloric acid.

The tetanus spores attached to the skin were killed after four weeks' exposure to the final lime bath.

These experiments indicate that gelatin derived from infected bones or skin which has been subjected to the above process will not contain living spores of anthrax or tetanus bacilli, if all parts of the tissues are equally exposed to the action of the various baths and to the heat.

RESULTS OF AGGLUTINATION TESTS FOR GLANDERS IN HORSES.

R. E. PICK, M. D., Assistant Bacteriologist.

During the past ten months the sera of 833 horses has been tested for the agglutination reaction for Glanders. These horses have been sent in under the various headings given in table No. 1. These headings bear no relation to the final classification, because it frequently happens that horses apparently normal will give a high agglutination reaction and on being subjected to the mallein test will give a reaction thus indicating the presence of glanders. We have been able to autopsy a few such horses and have found the characteristic lesions of glanders present (see next page).

TABLE I.*

Agglutination.	Suspected.		Exposed.		Normal.		Showing Symptoms of Some Disease.		Clinical Diagnosis, Glanders.		Total.	
	No.	Per Cent.	No.	Per Cent.	No.	Per Cent.	No.	Per Cent.	No.	Per Cent.	No.	Per Cent.
200.....	4	14.81	40	16.00	44	8.80	2	3.92	0	0.00	90	10.91
500.....	3	11.11	54	21.60	101	20.20	10	19.60	0	0.00	168	20.16
1,000.....	5	18.51	60	24.00	118	23.60	14	27.44	1	20.00	188	23.77
2,000.....	9	33.33	55	22.00	104	20.80	10	19.60	0	0.00	178	21.37
5,000.....	4	14.81	22	8.80	78	15.60	7	12.73	2	40.00	113	13.56
10,000.....	2	7.40	19	7.60	55	11.00	8	15.68	2	40.00	86	10.32
Total.....	27	3.24	250	30.01	500	60.02	51	6.12	5	0.6	Total, 833	Total, 100 per cent.

* The percentage in each column is calculated on the total number of animals in that particular class, thus: at 200 dilution we have 4 cases under the suspected division, or 14.81 per cent. of a total of 27 suspected cases. The percentages opposite the totals are calculated on the entire number of animals, namely: on 883.

Table No. 1 is chiefly interesting because it shows first the relation that the agglutination reaction bears to the clinical diagnosis, also that the greater number of cases of all classes react between the dilutions of 1:500 and 1:2000, thus making this range doubtful unless the mallein or clinical symptoms are present. The dilution of 1:1000 shows the greatest percentage of positive reactions, namely, 23 per cent. of the total of all the cases. It will also be noticed in this table that exceedingly few cases are reported with a clinical diagnosis of glanders; in fact, only 5 out of a total of 833 cases, or 0.6 per cent. of the total of cases.

Final reports could not be obtained for all horses tested, but in table No. 2, the results are given in 202 cases. The greatest number of cases

TABLE II.*

Table Giving Agglutinations, and Subsequent Data for 202 Horses in Which Such Data were Obtainable.

Agglutination Reaction.	Discharged or Working.		Mallein.***		Killed or Died from Glanders.		Total.	
	No.	Per Cent.	No.	Per Cent.	No.	Per Cent.	No.	Per Cent.
200.....	7	18.28	0	00.00	3	4.05	10	4.95
500.....	14	24.56	6	8.45	2	2.70	22	10.89
1,000.....	12	21.05	12	16.90	12	16.20	36	17.82
2,000.....	13	22.80	20	28.16	17	22.98	50	27.35
5,000.....	8	14.03	19	26.77	23	31.08	50	24.75
10,000.....	3	5.26	14	19.71	17	22.98	34	16.83
Total.....	57	28.22	71	35.14	74	36.63	{ 202 Cases. }	100

* Table No. 2 is to be read in the same manner as Table No. 1.

discharged or working show an agglutination index between 500 and 2,000, while the number reacting beyond this point diminish. On the other hand, horses reacting to mallein or those dying or destroyed because of glanders show a steady increase in agglutination reaction up to 5,000, with a slight diminution in numbers at 10,000. Even, at this index, however, the number is greatly in excess of those discharged or working, thus only 3 horses out of 34 showing an agglutination reaction of 1:10,000, gave no other evidence of the presence of glanders.

This, then, bears out the former conclusion that between the range of 500 and 2,000, the evidence of the existence of glanders based upon the agglutination reaction alone is less clear and definite, while the increase in the height of agglutination also increases many times the probability of glanders infection being present. It is also interesting to note that at 500 agglutination, 14 horses were discharged or working and 2 killed or died from glanders out of a total of 22; at 1,000 agglutination 12 discharged or working and 12 killed or died out of a total of 36; or an equal proportion of the two classes; at 2,000 agglutination 13 discharged or working and 17 killed or died, showing a tendency for this degree of agglutination to have some diagnostic value; at 5,000, 8 are discharged or working and 23 killed, showing a marked tendency of the test at this point to have positive diagnostic value.

The number of animals, in the above table, reacting 1:10,000 is hardly large enough to warrant analysis, though it is probable that agglutination at this dilution will have still greater diagnostic value.

THE SIGNIFICANCE AND MICROSCOPICAL DETERMINATION OF THE CELLULAR CONTENTS OF MILK.

ARTHUR I. KENDALL, Ph. D.

The tendency toward segregation of population which has been so marked during the last two decades has necessarily been accompanied by a parallel, retrograde movement of many important industries upon which the inhabitants depend for an essential portion of their daily ration. Perhaps the most noteworthy example of this is the milk supply.

The complex of difficulties which was great under the old regime, where the production was in the immediate neighborhood of consumption has been greatly magnified, and at the same new and intricate conditions have arisen which make the problem of a hygienic milk supply a very difficult one. New and more complete understanding of the laws relating to pure milk and the public health have placed an added responsibility upon the sanitary officials on the one hand to safeguard the populace from careless, ignorant or unscrupulous dairymen and at the same time has made it necessary for them to be able to act in harmony with the producers to enact measures governing the production and distribution.

In order to intelligently fulfill their duty as sanitary authorities, modern methods must be devised which shall become in their hands the tools by which they may determine from time to time the condition of the milk as it is produced, handled and sold.

Such methods need not have, nor, indeed, can they hope to attain the precision of ultra scientific measurements; in fact, proximate methods of analysis in this connection are of greater value; they are more rapid, and permit a greater volume of work in a unit time, a point of the greatest importance where large numbers of purely routine determinations must be made.

Special attention will be paid in this paper to the cellular content of milk-leucocytes (pus cells as they are sometimes called) and bacteria.

The Bacterial Content of Milk.

Bacteria occur in milk from three sources; from the udder tissues itself, from unclean surroundings of the cow, and from additions which may occur at any stage of the handling of milk till it is finally consumed.

Microorganisms in the udder, again, originate in two ways; the first by bacteria which gain access to the milk duct and cistern between milkings, and those that habitually occur in these portions of the secreting tissue. The first class, those derived from outside contamination and which multiply between milkings are, as a rule, harmless in the numbers voided, and vary in type from time to time, depending upon the environment of the cow. The second class also vary, but with a definite animal are apt to become in reality a "physiological flora" which persist in spite of outside conditions. Russell and Moore have found that practically no cow gives milk that is sterile; even the stripplings contain a few bacteria, and Moore and Ward have been partially successful in introducing definite species of certain organisms and establishing a semi-permanent flora. It should be noted, however, that bacteria growing in the udder tissue under these conditions is not wholly permanent, but have to be reintroduced from time to time.

Russell has shown that bacteria causing disagreeable taste to milk may become localized in this manner, and at times it is extremely difficult or impossible to remove such growths.

In diseased udders the picture, however, is radically different, and one finds a multitude of bacteria in milk which, under normal conditions would only contain a few bacteria.

Probably the most common organisms occurring in such cases is the streptococcus of mastitis, although Gaffky has isolated several strains of colon-like bacteria which are extremely virulent for experimental animals, and probably not without effect upon man.

The streptococcus of mastitis should be carefully distinguished from the ordinary milk streptococcus, which is very abundant in many samples of milk which have been allowed to stand at temperatures not below 45 degrees C. The former is a variant of streptococcus pyogenes, and has frequently enlarged elements which were called by Hueppe "arthrospores," and usually occurs in long chains. Furthermore the

elements are large, usually a micron or more in diameter, while the milk streptococcus is much smaller. The mastitis organism is associated with large numbers of poly-and mononuclear leucocytes, as well as cell detritus, and the whole mass interwoven with strands of fibrin. The intensely blue staining with methylene blue as well as the agglutinated appearance is distinctive.

Bacteria derived from an unclean environment of the cow-dirt, unclean milkers, dirty udders, and those from handling after the milk has left the farm may be almost infinite in their variety, and may contain under exceptional conditions several kinds of pathogenic bacteria.

Estimation of the Number of Bacteria in Milk.

There are two methods by which the number of bacteria may be estimated in milk, the "direct," or smear method, and the indirect method, in which the numbers of colonies developing upon solid nutrient media are taken as an index of the numbers of organisms originally present.

The latter method is inexact for several reasons, partly because it is impossible to break up groups or chains thoroughly in the sample; this is so because the milk is very viscous, and there is very little difference in specific gravity between the milk and bacteria; the second cause is the fact that by no means can one with our present methods cause all bacteria to develop in the same medium. The food conditions which are favorable for the majority are unsuited for the minority, and there is no reason, furthermore, for believing that the ratio of those growing to those not growing is constant.

As the result of this difficulty, attempts have been made to find a substitute for the plating method, and the smear method is the direct attempt in response to this need to determine the bacterial content.

There are three general modifications of the direct method; the measure of the sediment (Trommsdorff's method). This is in reality not a bacterial method, but expresses instead the volume of material which can be sedimented from a given volume of milk, and includes not only bacteria and leucocytes, but various other foreign material whose specific gravity is sufficient to bring it down during the process of centrifugalizing.

The Doane-Buckley method corresponds to the direct estimation of blood cells in the blood; the numbers of bacteria and leucocytes which may be counted, when a definite aliquot portion of the milk is placed in a blood counting cell multiplied by a suitable factor furnish evidence of the number of cells originally present.

The third and most practical method is the one first made practical by Stewart, of Philadelphia. The principal feature is the fact that bacteria derived from a definite, representative portion of milk are centrifugalized, the sediment is smeared upon a definite area, and representative definite portions of this area are counted with respect to cellular content, the numbers of cells multiplied by an appropriate factor, and the result gives the number of cells originally present.

Russell has greatly increased the accuracy of the procedure; he noticed that as the cream rises during centrifugalization, varying numbers of bacteria and leucocytes rose at the same time, due to a sort of "raft" action on the part of the fat globules. If, now, one heats the milk to 65–70 degrees C. before the sedimentation, the fat globules become very much less in size, due to a bursting of their envelopes, and there are only a few cells carried up with the fat under these conditions, an amount equal to less than four per cent., as a rule.

Various observers have from time attempted to establish a ratio between the plate count and the direct method, and while these factors may hold with a fair degree of constancy, this must not be taken to indicate in cases of variations from the ratio of duplicate samples in a small number of cases, that one or the other result is necessarily incorrect. A discussion of the reason for this has already been given above.

Pasteurized milk cannot be examined by the smear method for the number of living bacteria, because some of the bacteria killed by heat stain for the most part quite as well as those which have developed since the pasteurization.

In general, where rapid work and proximate results only are desired, the direct or stained smear method, carefully carried out may, with certain precautions, which will be described in detail in the succeeding portion, be used and relied upon to furnish a fair index of the bacterial content of milk. Good milk shows uniformly a low content

in bacteria (except pasteurized samples), while milk bacterially rich can be readily detected.

One of the greatest advantages of this method lies in the fact that samples may be preserved in their original form, without fear of bacterial increase by the addition of a small amount of formaldehyde. Larger amounts of aldehyde, however, cause coagulation of the samples.

Leucocytes in Milk.

The term "leucocyte" has been applied to a variety of cells which may occur in milk, including mono- and polymorphonuclear cells, gland cells, as well as cell detritus in certain instances. Many investigators assume that one cannot distinguish between a leucocyte and a gland cell; this is, however, as a rule, not difficult, because gland cells are, for the most part at least, mononuclear, while the majority of leucocytes are polymorphonuclear. The mononuclear leucocytes, however, may be thus confused.

Although the bacterial content of milk gives one an idea of the present condition of the milk, and in exceptional cases furnish information which may lead back to the dairy, herd or even cow responsible for the abnormality, yet it is extremely desirable to have some factor which shall indicate more specifically the condition of the animal which produced the milk; a factor which does not change after the milk is drawn, and the leucocytes seem to offer such a criterion.

It is evident that they do not multiply after they leave the cow, they are stable toward degeneration and staining reactions for long periods of time and in many ways they seem to be eminently suited for this purpose.

Many studies have been made with the view of determining their significance; attempts have been made to correlate their numbers with the leucocytic or erythrocytic content of the cow's blood, but no such relation can be shown to exist. Stokes and Wageforth showed that cows kept in sanitary conditions were much less likely to have high leucocyte counts in the milk than were those kept in unsanitary surroundings. Text books are silent upon what may be designated the normal or physiological leucocyte content of the blood, and the succeeding pages represent studies made for that purpose. It is known that im-

mediately before and after parturition the numbers may be very high; and legislation has prevented milk from being placed upon the market for a definite length of time after parturition.

Method of Procedure.

Before describing the method employed in this work in detail, it may be well to mention briefly the general plan followed with reference to the collection of samples. Milk was obtained from two farms which furnish "certified milk" of excellent quality to Greater New York. The samples were collected under the immediate supervision of the writer from cows that, so far as careful inspection could show, were undoubtedly normal in every respect. A few samples were taken from animals that had disease also, but these specimens will be discussed under appropriate headings, and are quite distinct from those from normal cows.

To determine the cellular content of milk, samples were carefully and thoroughly shaken, heated to 70 degrees C. for 10 minutes, again thoroughly shaken, and placed in centrifuge tubes of small bore, a cubic centimetre in each, revolved for 10 minutes at 2,400 revolutions, and spread upon slides in areas of two square centimeters each. The smears were then either fixed in the flame, or flooded with a mixture of absolute alcohol and ether, stained with methylene blue, dried and were then ready for examination.

Stewart uses circles having a definite area, and this method is to be preferred to squares of the same size, because the sediment can be distributed easier in a circle than a square, if one is forced to use the stopper of the centrifuge tube as the agent for distributing the bacteria, but for rapid work with many samples the difficulty of establishing accurate circles more than offsets any slight gain in accuracy. If one counts ten representative fields, using a low-power lens as a guide, the results, as shown by duplicate determinations, are strikingly harmonious.

For counting, the writer has used with great success a "net" micrometer, ruled into 100 squares, fitting into the eyepieces at the diaphragm so that the squares seem superimposed upon the microscopic field, and one sees a picture precisely like that seen in a blood counting cell. By regulating the length of draw tube, with the oil immersion

lens and a definite eyepiece, it was possible to so adjust the relation between magnification and size of squares so the area counted was exactly $1/10,000$ of a square centimetre. Inasmuch as the contents (cellular), of a cubic centimetre were spread upon two square centimetres, it was possible, by multiplying the average number of cells per field by 20,000 to determine with reasonable accuracy the number in the original sample.

The sources of error are:

- 1—Error in sampling.
- 2—Error in spreading on the slide.
- 3—Loss of cells by washing off, due to imperfect fixation methods.
- 4—Error of counting.

The error due to imperfect fixation is variable; careful comparisons of samples fixed with absolute alcohol and ether with the flame showed practically no difference. On the other hand, in almost every instance in which there were fairly large numbers of cells per field (70–200), examination of the water which was used to replace and remove the excess of stain showed a variable number of organisms; slides stained with methylene blue, but in which the stain was allowed to become dry, and examined without washing showed in such cases a rather higher content, and this observation is in accord with that of Hehewerth, who found a large percentage of bacteria removed by washing when they were fixed in the ordinary way. The losses in the milk slides, however, were much less than those reported by Heheworth, and this is undoubtedly due, in part at least, by the "fixatif" action of the milk proteids.

The spreading error has been commented upon, and there is no method of overcoming it except by a careful examination with a low power lens preparatory to counting, and rigid attention to the distribution in making the determinations.

The greater the total area counted, other things being equal, the greater accuracy the method; in practice, it is hardly possible to count more than ten representative fields, using the net micrometer, but experience has shown that agreement between averages so obtained in duplicate samples is rather less variable than those obtained by plating methods on the average. If, however,

cells per field, the agreement is less close, but here again there is parallelism with plate cultures. Where there are very few or very many colonies.

The advantages of the method are:

1. Both leucocytes and bacteria are counted. Frequently one may obtain valuable information about the past history of the milk and the cow which the bacterial count alone would utterly fail to detect.

2. Samples may be preserved in their original state by the addition of formaldehyde with no further increase of bacteria.

3. Examinations may be made the same day that the samples are taken, finished and recorded.

4. A rather definite idea of the condition of the dairy, herd or cow, as the case may be, may be obtained in daily samples; the exact value, however, of this information is at present uncertain.

5. A general idea of the presence or absence of certain types of bacteria present may be determined; this is of importance in many connections.

6. A much greater number of samples can often be analyzed by the smear method than are possible with the plating method, taking into consideration the preparation of media, sterilizing apparatus and space required.

7. While there is no means at present available to determine the accuracy of either the plating method or smear method, duplicate determinations made with the latter procedure as a rule vary within 20 per cent., provided there are not more than 70 per field, which is as close as is possible with bacterial methods in general.

The Leucocytes of Normal Cow's Milk.

Milk freshly drawn from 168 cows was examined by the smear method as described above, and over 80 per cent. showed less than 400,000 leucocytes per cubic centimetre of milk, or on an average of less than 20 per microscopic field. These cows were as far as a rigid inspection could show, free from any udder disease, and were normal in every respect. The fore milk, generally speaking, contained relatively a few less leucocytes than the strippings, but this difference was not marked nor constant. This fact is of importance in determining the numbers of leucocytes that may be allowed in market milk; the present

legal limits in those laboratories which have made careful studies of this subject are in close accord with this number, Boston, for example, insisting 500,000 or less as the permissible number.

What can be done in this respect by dairymen is shown by the following table; the numbers of leucocytes per field, multiplied by 200,000 will give the number per cubic centimetre.

A herd of 28 cows were chosen; they were in perfect health, very carefully groomed and hygienically housed.

Leucocytes Per Field.	Number of Cows.
1-10.....	22
11-20.....	2
21-30.....	2
31-50.....	2
above 50.....	none.

The period of lactation, that is, the time elapsing since the cow calved, seemed to make absolutely no difference in the leucocyte content, provided the samples were taken at least 10 days after parturition, after the cow had again become a normal producer. Cows that had been milked continuously for periods up to a year, and those that were only recently milked showed absolutely no difference in this respect. This statement is based upon the findings of the 168 cows referred to in Table I., and comparisons with a few cows from other herds seem to substantiate this statement.

TABLE I.

Table Showing the Numbers of Leucocytes per Field in the Milk of Normal and Diseased Cows.

Market milk cannot, theoretically, be compared directly with these statements; milk retailed in cities is obtained from a large number of cows, and one poor cow (high in leucocytes), would, generally speaking, make very little difference if that milk were mixed with the milk of a dozen normal cows. At the same time, the milk will attract attention, even in this case because the agglutinated appearance still persists, and one is very apt to find clumps of leucocytes, many of which may be phagocytic, which at once attract attention.

A few of the cows showed a rather high leucocyte content, in spite of the fact that they were apparently normal. The exact significance of this phenomenon is at present a mystery, and so far the writer has been unable to offer any definite explanation. It is possible that by carefully watching such animals, some infection might be noticed. It is interesting to note in this connection the fact that anything that tends to seriously disturb the animals may result in a definite increase in the numbers of leucocytes. The writer happened to be in a barn in which the animals were being tested with tuberculin; previously the cows had shown moderate counts, as a rule below 20 per field; at the time of the testing, the same animals (27), showed in over 80 per cent. of the cases a decided increase in leucocytes, not dependent upon the presence of a positive reaction, but apparently due to the unusual disturbance. After a week, those animals which were again examined, were back to their normal.

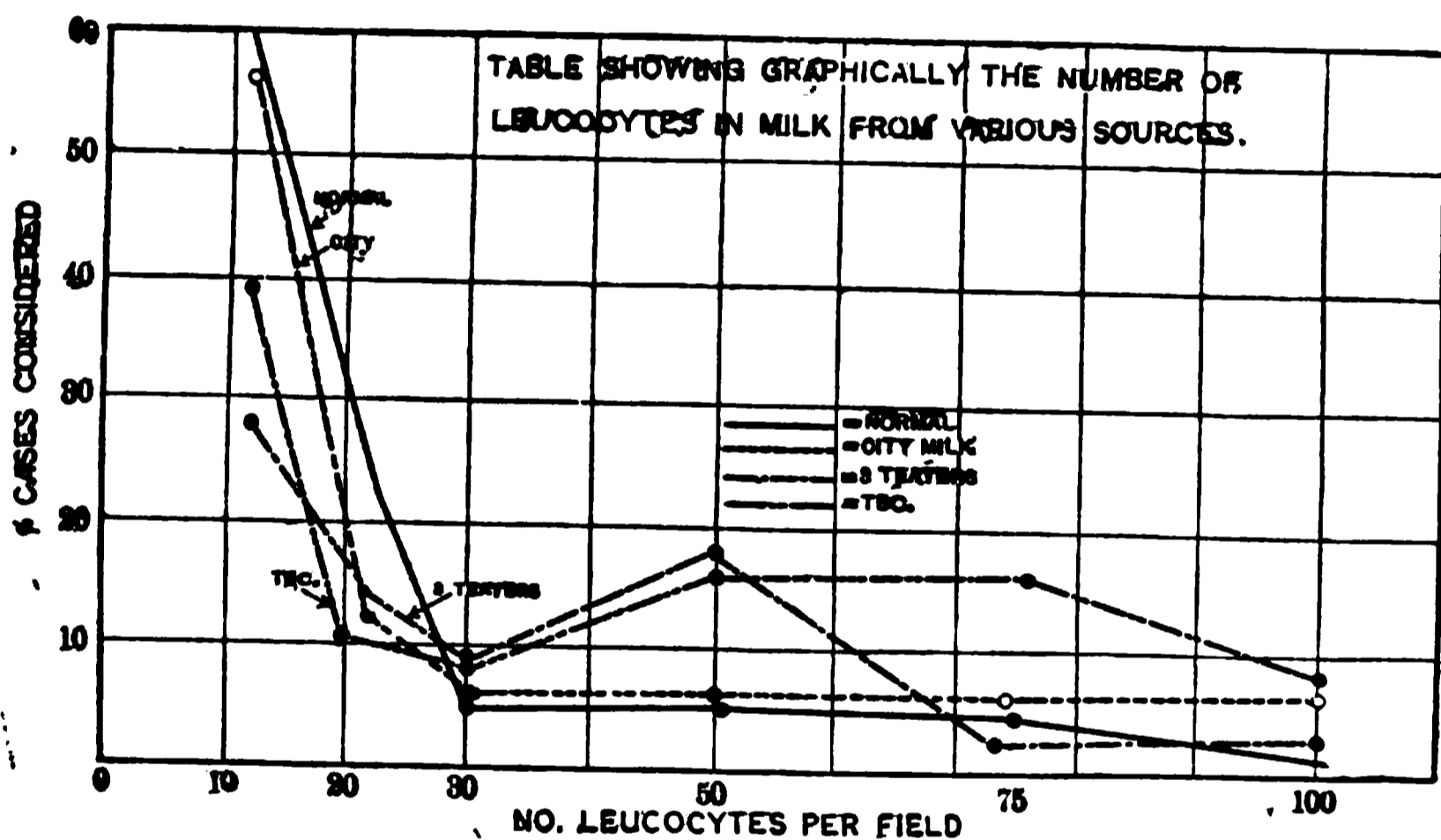
Milk from Cows Reacting to the Tuberculin Test.

The results are not as satisfactory as the preceding ones, because it was impossible to observe the cows at the time of milking to detect signs of udder disease which might influence the results, except in a few cases. In every sample, however, an tubercle bacilli could be detected by direct staining with the customary Ziehl-Neelson stain, or by the guinea pig test, using the sediment from 10 c.c. of milk. Table 1 shows the leucocytic content; the variations are greater by far than was the case with the normal cows. In certain of the samples it was possible to correlate the high leucocyte count with the presence of streptococci; the samples were fresh, and this fact, coupled with the fact that the cells were in many instances phagocytic, probably explains some of the abnormal results.

"Three-teaters."

Cows known as "three-teaters" have been the subject of discussion for some time; the trend of opinion has been to exclude them from the herd, and the results obtained in this work, based upon the examination of 89 cows certainly shows that in relatively large proportion of such animals there are an usually high number of leucocytes (see Table II.). The leucocytes are frequently phagocytic, and in many cases associated with streptococci, rarely with bacilli.

TABLE II.



It is interesting to note in this connection that Kitt, Guillebeau, Lucet and Streit have isolated colon-like bacilli from cases of mastitis; in some cases recovery was attended, apparently by occlusion of one or more teats. The organisms agreed in important details, furthermore with the colon bacillus, and it is extremely suggestive to compare these results with those of Jackson and others who have found colon bacilli by presumptive tests in milk, obtained from the city supplies.

In general, while a rather large proportion of three teaters may show abnormal numbers of leucocytes in their milk, it would be an injustice to arbitrarily exclude all such animals from the herds by law. Over 40 per cent. of the cases reported in this paper were quite as normal in this respect as the well kept cows of excellent dairies. It is not dif-

ficult to determine whether or no the inflammatory process, in those animals which have suffered from mastitis, had disappeared; and certainly congenital three-teaters have no inherent abnormalities, except a lack of complete development in the udder.

City Milk.

In all, 87 samples of city milk were examined in order to determine how, in general, mixed milk from a great variety of sources compared with milk from normal, individual cows. In no sense was the milk obtained for this purpose; it represents samples precisely similar in every respect to those sold every day in New York from the smaller shops and stores in small amounts.

By referring to the table it will be seen that about 66 per cent. compares very favorably with that derived from normal cows; where the animals are carefully housed and groomed. At the same time there is rather a large proportion which is not so clean. In the sediments one may actually see bits of hair, pieces of manure and amorphous matter. When one realizes that there is a possibility that such milk was made up of a few bad samples with a larger proportion of good milk, the significance of the microscopical examination in such cases becomes patent. In such milk as would under such conditions contain from 100 to 200 leucocytes per field, there are almost always very striking indications of abnormalities on the part of the cow; phagocytic cells are not rare, one sees streptococci of the larger types and in general there is a much more intensely staining reaction.

The bearing of this work upon the control of municipal supplies is the chief *raison d'être* for its initiation, and if the results which are represented above are not influenced by seasonal factors which, of necessity, could not for obvious reasons be taken into account, there is reason to believe that it is feasible to require dairies to furnish milk containing not more than 400,000, or at the most, 500,000 leucocytes per cubic centimetre. That over 80 per cent. of the cows in well-kept farms make this record is not new, and even the fact that a large percentage of regular city milk is of this quality shows that such a standard would not be a very great hardship to place upon the producer.

Summary.

1. The smeared sediment method of examining milk is rapid, practical and reasonably accurate.
2. Certain precautions must be employed in making such determinations.
3. Normal cow's milk in over 80 per cent. of the cases examined showed less than 20 leucocytes per microscopical field, or 400,000 per cubic centimetre.
4. This number of leucocytes is proposed as the legal limit in milk sold for human consumption.
5. Cows having mastitis or other udder disease should be rigidly excluded from herds, not only because they give diseased milk, but because there is danger of the disease being transmitted from cow to cow by milking.

A COMPARATIVE STUDY OF THE DIRECT MICROSCOPICAL AND PLATING METHODS FOR THE BACTERIOLOGICAL EXAMINATION OF MILK.

By HARRIET L. WILCOX.

The following work was undertaken in order to compare the smear with the plate method for the bacteriological examination of milk. Of the 183 samples examined, all with one or two exceptions were sent in by the Department of Health Milk Inspectors and regarded as "ordinary milk."

The technique used was essentially that of Stewart, and was as follows: After the milk had been thoroughly shaken fifty times, one c.c. was pipetted off from each sample and placed in a small glass tube, three inches in length and one-quarter of an inch in diameter and closed at either end with rubber corks. The tubes were then centrifugalized for 10 minutes, after which the upper cork was removed and the supernatant milk and cream were gently poured off. The lower cork upon which the sediment had been precipitated was then removed, and rubbed as evenly as possible on a slide over an area of 2 square centimeters upon which a drop of sterile water had been previously placed. The smears having been allowed to dry in the air were fixed with methyl alcohol, and stained for a few seconds in a watery solution of methylene blue.

It was found that there was no loss in the milk smears when the slides were fixed in this way, while in every case where the smears were fixed by heating, which necessitated washing the slides to remove the excess stain, the wash water was seen to have a decidedly milky appearance, and smears made from it showed that a considerable percentage of bacteria had been washed from the milk smears.

Russell found that by heating the milk from 65–70 degrees C. the fat globules were diminished in size, due to the bursting of the envelopes and that only a few cells were carried up with the cream during centrifugalization; it was thought that this would hold true in regard to the bacteria, so that from each sample examined duplicate smears were made from a portion of the milk which had been previously heated from 65–70 degrees C. for 10 minutes.

To estimate the number of bacteria present, ten fields of each smear were counted, averaged, and multiplied by 20,000, inasmuch as the net micrometer used in conjunction with the 1/12 oil immersion gave a field equal to 1/10,000 of a square centimeter, and that the bacterial content of one cubic centimeter was spread over an area of two square centimeters. Two counts were made from each smear, in the first the bacteria, if in clumps, were regarded as such, while in the second the number of individual organisms was estimated as nearly as possible. The two counts made from the smear were then compared with the bacterial count obtained by plating.

TABLE I.

Unheated Milk (direct count).			Milk Heated from 65°-70° C. (direct count).		
Number of Samples.	Bacteria (group).	Bacteria (Indiv.)	Bacteria (group).	Bacteria (Indiv.)	Colonies in Nutrient Agar, Plated.
197	2,316,000	8,820,000	420,000	4,134,000	7,360,000
1,152	1,746,000	6,018,000	966,000	5,268,000	5,240,000
1,965	3,186,000	11,976,000	330,000	3,702,000	3,870,000
1,249	3,252,000	9,192,000	360,000	2,088,000	2,670,000
22	7,596,000	31,740,000	900,000	5,502,000	6,000,000
1,955	7,536,000	18,828,000	1,074,000	21,732,000	5,844,000
231	3,120,000	13,002,000	312,000	4,644,000	3,760,000
1,858	2,364,000	6,204,000	186,000	1,944,000	2,950,000
10	3,384,000	22,680,000	1,032,000	11,862,000	4,600,000
1,860	2,586,000	24,444,000	948,000	17,076,000	9,700,000

It being impracticable to tabulate the counts of all the milk examined, the above ten samples were chosen as representing fairly the total results obtained.

By a comparison of the results obtained by direct method with those of plating (Table I.), it is difficult to draw any conclusions as to which is the more valuable method. In some milks where the bacteria are well distributed and not in chains or clumps the individual bacterial counts from the smears compare very closely with those from the plates, while in other milks the group bacterial counts agree much more closely with the plate counts and it is just here that frequently a great inaccuracy in milk plating is strikingly brought out, *e. g.*, the number of colonies in a

milk plate is regarded as representing the number of bacteria in a given sample of milk; in some milks, however, the organisms are held together in chains or masses which are not easily broken up by shaking, and a colony may, therefore, represent a group composed of from one to two hundred or more bacteria, thus giving an erroneous estimate of the actual number present.

On the other hand, in counting only a limited number of fields in a smear, there may be as great a discrepancy in the resulting counts as in milk plating. For example, the following duplicate counts were made from five smears, the fields in both counts being taken as nearly as possible from the same part of the smear.

Number of Samples.	Bacteria.	
	Original Count.	Duplicate Count.
467	38,000	272,000
605	104,000	270,000
382	642,000	338,000
397	32,000	152,000
543	532,000	510,000

An advantage of the smear method is that it is possible to estimate not only the number, but to some extent the different varieties of organisms, within two hours after the arrival of the milk at the laboratory.

In regard to heating the milk to 70 degrees C. for 10 minutes before centrifugalization to obtain a higher and more accurate count of the bacteria present the results have been most disappointing. The comparison of the counts made from the heated samples of milk with those of the unheated shows that in 25 per cent. we get a higher bacterial count on the average in the unheated milk (see Table I.). Although a comparative count of the leucocytes was not made, a great increase in their number was very noticeable in those smears made from the heated milk.

To determine just what proportion of bacteria in one cubic centimeter was thrown down by sedimentation, the samples of milk were centrifugalized after which plates were made from the cream, middle

portion and sediment respectively. The plates were then incubated at 27 degrees C. and counted after 72 hours. The results showed that the bacterial count of the cream and middle portion together was, on the average, twice as high as that from the sediment alone.

For routine work it would scarcely be practicable to count the individual organisms in each smear, but after a little training one could estimate approximately the number of bacteria present. If the smear showed on the average two or less organisms per field, the bacterial count for that sample would be less than 50,000 organisms per c.c., and might, therefore, be considered of good quality. When the microscopic field was filled with a hundred or more bacteria or large clumps comprised of several hundred bacteria, it is clearly evident that the milk contains 1,000,000 or more organisms per c.c., and would be regarded as poor milk. The smears from milk between these two extremes, however, might require a more accurate counting, or it might even be advisable to plate.

Conclusions.

1. The smear or direct method for examining milk as it takes but a few hours is much more rapid than the plate method.

2. The determination of the different morphological varieties of organisms and also the cellular content of the milk is possible by this method.

3. To make a leucocytic count the milk should be heated to 70 degrees C. for ten minutes; for bacterial count, the raw milk gives better results.

4. After centrifugalization for ten minutes, about one-third of the total number of bacteria in one cubic centimeter were thrown down, the rest being suspended in the supernatant milk and cream. The bacteria seen in the smear represent, therefore, only about 33 per cent. of the total number of the bacteria in the milk.

5. For routine work, it is not necessary to make an accurate count of the bacteria, but merely to examine the slide as a whole and estimate the number present.

6. That by employing either the plate or smear method the results thus obtained are only approximate.

THE TIME AND TEMPERATURE FACTORS IN THE BACTERIOLOGICAL EXAMINATION OF MILK.

HARRIET L. WILCOX.

I. Temperature Most Advantageous for the Growth of Milk Bacteria.

There are varied opinions among laboratory workers as to what is the most suitable temperature for the incubation of milk plates. Some believe that the maximum growth of the more important bacteria is obtained when the plates are grown at 37 degrees C., while others are in favor of room temperature, as giving a more reliable count. To determine this point as nearly as possible, plates were made from certified and ordinary milk and were grown at 37 degrees C., 27 degrees C. and room temperature, the latter averaging about 23 degrees C. The number of bacteria in the duplicate plates grown at the above temperatures was counted and averaged, the plates incubated at 37 degrees C. and 27 degrees C., being counted for four consecutive days, while those grown at room temperature were counted from the second to the seventh day, inclusive. In the 17 certified milk samples examined, 10 or 58 per cent. showed on the average twice as high a count when grown at room temperature (Table I.); the results in the ordinary milk were even more striking, 52 per cent. of the samples having shown on the average nine times the number of organisms in the plates grown at room temperature than those at 37 degrees C. (Table II.). In the second experiment, ordinary market milk alone (both unheated and heated), was used and plates made from it were grown at 37 degrees C., 27 degrees C. and room temperature. The maximum growth was obtained at 27 degrees C., in 65+ per cent. of the samples tested, while those at the room temperature had the greatest number of bacteria in 26 per cent., thus leaving only a small proportion having the maximum growth at 37 degrees C. (Table III.). The two samples showing a slightly greater number of colonies at 37 degrees were "pasteurized" milk. This previous heating in destroying the non-spore bearing varieties undoubtedly was the cause of this variation in these samples from all of those not so treated.

II. *Summary and Conclusions Upon the Time and Temperature Most Suitable for the Bacterial Count of Milk.*

In the appended tables it will be noticed that in every case the highest count is at the end of the fourth day or later, without regard to which temperature was used. The difference, however, is not sufficient to make it advisable to delay beyond a certain length of time. It is noticed that at 37 degrees there is a marked increase in the number of colonies at the end of the second day over the first, and then only a moderate increase for each successive 24 hours. Probably all would agree that when subjected to 37 degrees, 48 hours is a sufficient time to allow for growth. It is true that another day will allow of something like 10 per cent. additional growth, and a fourth day another slight increase, but this is too little to make such a delay advisable.

At 27 degrees there is also a great increase between the count at the end of 24 hours and at the end of 48 hours. There is a still considerable increase in the next 24 hours, and a smaller one at the end of the fourth day. Probably most would choose either a growth for 48 hours or for 72 hours. Where the delay is not important 72 hours should be regarded as the best time to allow for a growth. Here, as at 37 degrees, it is to be remembered that a considerable increase will take place if a longer time is allowed for the development of colonies. At 23 degrees there is even a greater difference between the growth at the end of 24 hours and at 48 hours, and a slighter greater difference at the end of 72 and 96 hours. Here, probably, most would agree that either a three-day or four-day period of growth be allowed, three days giving a practical time limit in our opinion.

When we consider the temperature at which milk bacteria should be allowed to develop, the enormous difference between the counts of colonies developing at 37 degrees and at 27 degrees or lower is at once apparent. At the lower temperatures from ordinary market milk from two to ten times as many colonies develop, and from purer milk a slightly less excess. It seems to us, therefore, hardly to be open to argument that one of the lower temperatures should be advised. Between 27 degrees, 23 degrees and still lower temperatures down to 20 degrees there seems to be but little choice. If it is desirable to have

always the same temperature we should advise one of 27 degrees, because this is easy to obtain the entire year and also because it gives a somewhat more rapid growth. It seems, however, justifiable from our results to allow the colonies to develop at any temperature between 23 degrees and 27 degrees, simply allowing a day longer for those growing at 23 degrees than those at 27 degrees.

TABLE I.

Certified Milk. Name of Sample.	Average Counts from 2 Plates Grown at 37° C.	Average Counts from 2 Plates Grown at R. T., 23° C.
R. G. F.....	1,800 colonies per c.c.	1,550 colonies per c.c.
Meadowedge.....	350 " "	700 " "
Gedney.....	2,450 " "	350 " "
Brookside.....	800 " "	1,250 " "
White Clover.....	4,250 " "	6,300 " "
Eralaust.....	250 " "	1,350 " "
Gedney*	250 " "	250 " "
Meadowedge.....	8,500 " "	5,900 " "
Brookside	1,600 " "	1,200 " "
Briarcliff	5,150 " "	1,800 " "
M. M. F.....	10,200 " "	11,050 " "
K. G. F.....	1,200 " "	2,250 " "
Brookside	250 " "	700 " "
Briarcliff	3,500 " "	4,200 " "
Gedney.....	1,650 " "	2,250 " "
Locust.....	21,550 " "	67,500 " "
Thorndale.....	1,100 " "	650 " "
Total.....	64,850 " "	109,250 " "
Averages.....	3,814+ " "	6,426 " "

* This sample is disregarded in the calculations as the counts at the different temperatures are the same.

TABLE II.

Ordinary Milk. Number of Samples.	Average Counts from 2 Plates Grown at 37° C.	Average Counts from 2 Plates Grown at R. T. 23° C.
204	80,000 colonies per c.c.	5,000 colonies per c.c.
878	20,000 " "	40,000 " "
1,383	20,000 " "	35,000 " "
1,521	55,000 " "	15,000 " "
1,557	55,000 " "	20,000 " "
1,286	120,000 " "	40,000 " "
1,334	160,000 " "	100,000 " "
1,705	25,000 " "	20,000 " "
1,892	45,000 " "	55,000 " "
2,028	20,000 " "	75,000 " "
707	25,000 " "	30,000 " "
737	230,000 " "	615,000 " "
988	455,000 " "	70,000 " "
1,516	3,370,000 " "	995,000 " "
1,891	30,000 " "	20,000 " "
934	245,000 " "	2,655,000 " "
1,051	15,000 " "	575,000 " "
1,128	60,000 " "	175,000 " "
1,281	360,000 " "	5,250,000 " "
Totals	5,390,000 " "	10,790,000 " "
Averages	283,684 " "	567,894 " "

TABLE III.

Counts from Plates Grown for Four Days.

Ordinary Milk. Number of Sample.	Counts from Plates Grown at 37° C.	Counts from Plates Grown at 27° C.	Counts from Plates Grown at Room Temp.
	Colonies per c.c.	Colonies per c.c.	Colonies per c.c.
847	60,000	705,000	385,000
1,097	100,000	325,000	135,000
1,200	415,000	7,495,000	6,800,000
1,400	1,200,000	10,220,000	10,590,000
1,596	255,000	1,375,000	790,000
356	210,000	405,000	270,000
696	2,425,000	8,690,000	6,510,000
*992	65,000	150,000	140,000
*1,014	85,000	45,000	50,000
942	2,245,000	5,030,000	11,295,000
1,346	3,440,000	12,115,000	7,675,000
1,658	60,000	3,170,000	3,250,000
2,019	2,000,000	2,910,000	2,735,000
*362	40,500	18,000	26,000
*1,034	2,500	21,000	16,000
*1,142	11,500	18,500	16,000
*1,151	8,000	19,500	31,000
*1,199	14,500	31,500	28,000
918	155,000	235,000	170,000
1,152	300,000	1,265,000	1,930,000
1,191	365,000	2,810,000	3,335,000
1,286	220,000	2,990,000	2,855,000
1,924	100,000	2,080,000	1,880,000
Totals.. .. .	13,770,000	62,912,000	60,912,000

* Pasteurized Milk.

Taking the number of colonies obtained at 27 degrees C. as a basis it was found that 98 per cent. of the colonies developed at 23 degrees C. as compared with 22 per cent. of colonies developing at 37 degrees C.

TABLE IV.

Certified Milk; Dilution, 1-100.	37°C.				23°C.						
	Colonies per c.c.				Colonies per c.c.						
	1st Day.	2d Day.	3d Day.	4th Day.	1st Day.	2d Day.	3d Day.	4th Day.	5th Day.	6th Day.	7th Day.
Brookside.....	175	250	250	250	Not counted.	300	450	450	450	700	700
Briarcliff.....	2,650	3,250	3,450	3,500	"	2,150	3,250	3,450	3,900	3,900	4,200
Gedney.....	900	1,400	1,450	1,650	"	850	1,650	1,800	2,000	2,100	2,250
Locust.....	10,900	16,150	20,000	21,550	"	48,650	58,600	60,050	62,800	65,850	67,500
Thorndale.....	650	900	1,000	1,100	"	250	400	450	600	600	650
R. G. F.....	750	1,600	1,800	1,800	"	250	450	Sunday.	1,100	1,450	1,550
Meadowedge.....	200	250	300	350	"	125	350	"	600	650	700
Gedney.....	950	2,150	2,400	2,450	"	75	150	"	250	300	350
Brookside.....	550	700	700	800	"	150	600	"	1,000	1,100	1,250
White Clover.....	1,700	3,100	3,950	4,250	"	250	1,950	5,300	6,000	6,300
Brookside.....	900	1,550	1,600	1,600	300	350	1,000	1,100	1,200
Briarcliff Herd.....	3,250	4,700	4,950	5,150	100	750	1,450	1,800	1,800
M. M. F.....	8,250	10,000	10,200	10,200	2,500	7,750	9,750	10,750	11,050
Meadowedge.....	4,800	7,500	7,900	8,500	1,100	2,300	4,200	5,250	5,900
R. G. F.....	550	1,000	1,200	1,200	900	1,450	2,150	2,200	2,250
Totals.....	37,175	54,500	61,150	64,350	57,950	80,450	{ 87,000 Est. }	96,550	103,750	107,650

TABLE V.

Ordinary Milk, Number of Samples.	37° C. Dilution = 1/10,000.					27° C. Dilution = 1/10,000.					23° C. Dilution = 1/10,000.				
	Average Counts.					Average Counts.					Average Counts.				
	Colonies per c.c.					Colonies per c.c.					Colonies per c.c.				
	1st Day.	2d Day.	3d Day.	4th Day.		1st Day.	2d Day.	3d Day.	4th Day.		1st Day.	2d Day.	3d Day.	4th Day.	
847	30,000	60,000	60,000	60,000		130,000	635,000	670,000	705,000		55,000	315,000	375,000	385,000	
1,097	30,000	75,000	100,000	100,000		105,000	270,000	325,000	325,000		1,000	105,000	120,000	135,000	
1,200	235,000	395,000	405,000	415,000		2,945,000	7,080,000	7,445,000	7,495,000		2,125,000	6,110,000	6,675,000	6,800,000	
1,400	675,000	1,005,000	1,105,000	1,200,000		4,085,000	9,060,000	9,915,000	10,220,000		2,460,000	8,705,000	9,535,000	10,590,000	
1,596	75,000	145,000	230,000	255,000		395,000	890,000	1,325,000	1,375,000		45,000	610,000	710,000	790,000	
315	15,000	85,000	90,000	90,000		60,000	150,000	205,000	240,000		77,500	135,000	150,000	175,000	
872	15,000	45,000	50,000	55,000		45,000	100,000	110,000	120,000		5,000	75,000	105,000	115,000	
1,025	5,000	55,000	65,000	70,000		15,000	70,000	155,000	180,000		5,000	75,000	130,000	175,000	
1,782	30,000	30,000	30,000	40,000		15,000	30,000	60,000	70,000		15,000	25,000	35,000	45,000	
2,019	40,000	50,000	50,000	55,000		5,000	15,000	15,000	20,000		5,000	30,000	45,000	70,000	
942	Holiday.	1,530,000	2,005,000	2,245,000		Holiday.	4,530,000	4,800,000	5,030,000		Holiday.	9,010,000	10,530,000	11,205,000	
1,346	"	3,040,000	3,180,000	3,440,000		"	11,005,000	11,860,000	12,115,000		"	6,470,000	7,335,000	7,675,000	
1,658	"	40,000	55,000	60,000		"	2,883,000	2,995,000	3,170,000		"	2,885,000	3,175,000	3,250,000	
1,958	"	2,935,000	3,090,000	3,270,000		"	13,630,000	14,370,000	15,630,000		"	14,025,000	16,810,000	17,680,000	
2,019	"	1,925,000	1,930,000	2,000,000		"	2,785,000	2,850,000	2,910,000		"	2,590,000	2,715,000	2,735,000	
Total counts of the first ten samples for the four days.....						1,150,000	1,945,000	2,185,000	2,340,000		4,793,000	16,185,000	17,880,000	19,280,000	
Total counts of fifteen samples for the four days, the counts of the last five samples being estimated not counted for the first day.....						6,930,000	11,415,000	12,445,000	13,355,000		15,150,000	51,165,000	58,445,000	61,915,000	

REPORT OF THE BACTERIOLOGICAL EXAMINATION OF A TYPHOID CARRIER.

By DR. MARY E. GOODWIN and W. CAREY NOBLE.

On March 20, 1907, Mary M., a cook, was sent to the Detention Hospital to have her feces and urine examined for typhoid bacilli. She was suspected by Dr. Soper¹ of being the cause of twenty-four cases of typhoid fever in nine families for whom she had worked during a period of five years. The patient seemed to be perfectly well and denied having had typhoid. Her statement, however, is open to question.

On her admission, Conradi-Drigalski plates were made from her feces, and agar plates and broth cultures were made from her urine. The following day the Conradi-Drigalski plates showed about 80 per cent. typhoid-like colonies. Twenty-five of these colonies, which were all the colonies there were in a small area, were fished into broth and tested for agglutination with a high-grade anti-typhoid horse serum. All seemed to be the same organism and agglutinated in dilutions of 1-10,000, 1-20,000 and 1-40,000, while the controls remained actively motile.

Two of these cultures were carried out on the following media: broth, slant agar, stick agar, litmus milk, glucose-peptone-water fermentation tubes, neutral-red lactose peptone water fermentation tubes, gelatin stab, and Dunham's peptone solutions, tests for indol being made on the third and the seventh days. On all media their behavior was the same as that of the laboratory typhoid culture (Mt. S.), used as control. The following tables show the results of the agglutination tests compared with those of the laboratory typhoid culture and a laboratory colon culture.

¹ Journ. Amer. M. Ass. 1907-XLVIII, 2019-2022.

TABLE I.

Antityphoid Horse Serum.				Mary M.'s Serum.		
	I-10,000	I-20,000	I-40,000	I-100	I-200	I-500
Mt. S.	++	+I	+	+	+	—
Mary No. 1.....	++	+I	..	±	±	—
Mary No. 2.....	++	++	+I	++	+	±
Colon.....	—	—	—	—	—	—

++ = Absolute agglutination.

+I = Complete.

+ = Agglutination.

± = Fair agglutination.

TABLE II.

Absorption of Agglutinins.

Antityphoid Horse Serum. Absorption by Mt. S.			Antityphoid Horse Serum. Absorption with Mary No. 2.	
Dilution of Filtrate.	1-100.	1-200.	1-100.	1-200.
Mt. S.....	—	—	—	—
Mary No. 2.....	—	—	—	—
Colon	—	—	—	—

The agar plates and broth cultures inoculated with urine showed no typhoid. Three more specimens of urine were taken later and examined as before, but no typhoid bacilli were found.

The feces have been examined on an average of three times a week from March 20, 1907, to November 16, 1907. In only a comparative few did we fail to find the bacilli. During the summer months, July, August and September, we noticed that the plates contained only a few typhoid-like colonies, about one or two. In July five consecutive negative tests were followed by one showing about 40 per cent. typhoid-like colonies. During August, the stools showed no typhoid. In early September we began to find typhoid again, but only one or two colonies appeared on a plate. From September 11, 1907, to October 14, 1907, the feces again failed to show any typhoid bacilli. During this time the patient's diet was carefully regulated and she was receiving mild laxatives. On October 16, 1907, we made a very thorough test of the feces.

Six broth tubes were inoculated with the feces and from each of these three Conradi-Drigalski plates were inoculated. The following day all the eighteen plates showed about an equal number of typhoid-like colonies, each containing about 75 per cent. Thirty-six fishings were made into broth and tested with antityphoid horse serum in dilutions of 1-10,000 and 1-20,000. All gave a complete agglutination in both dilutions, while the controls remained actively motile.

Since October 16, 1907, weekly examinations of the stools have been made up to February 5, 1908, and with only two exceptions we have found from 25 per cent. to 50 per cent. typhoid-like colonies on the plates. These two exceptions were on November 13, 1907, and December 4, 1907, when no typhoid was found. On February 5, 1908, the last examination made up to present date, the plates contained fully 95 per cent. typhoid-like bacilli.

Urotropin has been given to the patient in 7-grain tabloids three times a day for the last three weeks; that is, since January 18, 1908. The three stools examined since the beginning of this treatment have all contained large numbers of typhoid bacilli.

A typhoid culture from Mary M. was tested for its resistance in sterile Croton tap water. It lived six days. On April 6, 1907, when we obtained almost a pure culture of typhoid from the stool, we inoculated the earth out of doors with some of the feces. The spot was carefully protected, but the following day the plates inoculated with the soil failed to show any typhoid bacilli.

Literature (typhoid).

1. Von Drigalski and Conradi H. in "Zeitschr. f. Hyg. und Infect.," vol. 39, 1902, pp. 281-300, state that they found typhoid bacilli in the stools of four persons who had no typhoid symptoms, but were in contact with typhoid patients.

2. Koch, R., in "Die Bekämpfung des Typhus," in "Veröffentlichungen aus dem Gebiete des Militär-sanitätswesen," pp. 20-24, says that in 32 cases of typhoid in which the stools were carefully examined and the organisms carefully isolated he failed to find typhoid bacillus in a single case after three months. He considers the typhoid bacillus an obligatory parasite for man and believes that water-borne typhoid is very much overestimated.

3. Frosch, B., in *Festschrift zum sechzigsten Geburtstag von Robert Koch*, pp. 691-703, suggests that houses in which cases of typhoid developed one after another where there seem to be no traceable outside sources of infection contain, in all probability, typhoid carriers. He does not, however, prove this by the examination of stools.

4. Dönitz, W., in *Festschrift zum sechzigsten Geburtstag von Robert Koch*, pp. 297-314, states that in tracing the source of infection in 122 cases of typhoid reported in Berlin during a period of six months, 39 of which proved not to be typhoid, 37 were due to direct contagion or to infected water or milk, and 83 were of doubtful origin. Included in the 83 he reports the following cases:

(a) A woman had typhoid after a visit in the country. In November she was dismissed from the hospital as cured. The following June her husband was taken sick with typhoid. An examination was then made of the wife's urine and typhoid bacilli were found. It was assumed, therefore, that they had been present during the entire seven months and had finally led to the infection of her husband. During this entire interval the woman was seemingly healthy.

(b) A similar case occurred in Basdorf. Two children were apparently infected by the mother who, though not sick herself, proved to have typhoid bacilli in her urine. It should be stated, however, that the surroundings were not clean, and that four months earlier there had been a case of typhoid on the premises. It was from this case that the mother became infected. Typhoid bacilli were also isolated from the ground in the yard where feces had been thrown.

5. Seige, in the "*Klinisches Jahrbuch*," 1905, Bd. 14, pp. 507-516, reports finding typhoid in the stools of a person one year after having typhoid, and in the stools of another person ten years after having typhoid.

6. Besserer, A., and Jaffe, J., "*Deutsche Med. Wochenschr.*," 1905, pp. 2044-2047," found typhoid in the feces of the following cases:

Tamble—Patient well—typhoid 4 years before.

No. 52—Patient well—typhoid 7 years before.

No. 2—Patient well—typhoid 3 months before.

No. 25—Same patient as No. 2—typhoid 6 months.

No. 78—Patient well—never had typhoid.

7. Levy, E., and Kayser, H., in the *Münch. Med. Wochenschr.*, No. 50, 1906, report the case of a woman 49 years old who had had typhoid in 1903, and had made a good recovery. In 1905-1906 she was suspected of causing several cases of typhoid, and examination of her feces demonstrated the presence of the bacilli. Later in 1906 the woman died, but not from typhoid, and an autopsy 19 hours after death showed the presence of typhoid bacilli in the spleen, the liver, the bile, in the wall of the gall bladder and inside the gall stones.

8. Dr. P. Klinger, in *Arbeiten a. d. Kaiserlichen Gesundheitsamte*, Bd. 24, p. 91, reports 23 typhoid carriers in cases under observation during the period from July 1, 1903, to March, 1905. Nine were males and fourteen were females. Eleven of the twenty-three were found while examining the stools of 1,700 well people who had never had typhoid. The other eleven were typhoid convalescents in whom the bacilli had persisted from three months to thirty years. Klinger said that "The plates showed from a single colony to almost a pure culture of typhoid. Sometimes 1 or 2 negative tests would be followed by one showing enormous numbers of typhoid."

9. Kayser, Heinrich, *Arbeiten aus dem Kaiserlichen Gesundheitsamte*, Vol. 24, p. 176, reports that 13.5 per cent. of the cases of typhoid in Strassburg in 1904-5 were traced to six typhoid carriers. These six typhoid carriers, all women, gave histories of having typhoid. One had had typhoid one year before, one 6 months before, another 10 years, another 24 years, another 27 years, and another 30 years before.

In all the cases the stools and urine were examined. In one case, that of a woman who had had typhoid 27 years before, the bacilli were not found. She was suffering from gall stones, however, and a test of her serum gave a positive Widal reaction.

10. Lentz. "Ueber Chronische Typhus Bacillen-träger, (*Klinisches Jahrbuch*, xiv., 1905, pp. 475-494), says that seven doctors have reported the following typhoid carriers: the first reports a case of one who had had typhoid three years before, who was known to cause two cases of typhoid; the second reports four typhoid carriers, who had had typhoid as long ago as 42 years, 15 years, 13 years and 12 years, respectively. To these four were traced 12 cases of typhoid. The third

reports a typhoid carrier who had typhoid 19 years before, who caused 6 cases of typhoid. The fourth reports a carrier who had had typhoid 1½ years before, but to whom no cases had been traced; the fifth had a case which had had typhoid 17 years before and had caused 27 cases; the sixth had a case who had had typhoid 10 years before and had caused one other case; and the seventh doctor reports a carrier who had had the disease 17 years before. To this last, two cases were due.

Out of 400 typhoid patients Lentz found that six retained the bacilli from periods ranging from 3 1/3 to 13 months.

When the bacilli were in the stools of typhoid carriers, Lentz found that he could not get rid of them by any treatment. Their retention is due, he thinks, to faulty metabolism and concomitant chronic disease. Faulty care during convalescence may also be a cause. He notes the predominance of women who are carriers, over the men, and especially married women who have borne children. In most cases, the bacilli are present in great numbers. Lentz suggests that the gall bladder may not be the only source, but that the appendix and the deeper folds of the intestines may also be involved.

In conclusion, he suggests the following rules for controlling these carriers:

1. Disinfection of stools.
2. Disinfection of privies.
3. Police notification.
4. Bacteriological examination of stools.
5. Prevention of any occupation where the carrier is in a position to infect others.

11. Goldberger (1907) has an excellent bibliographical and statistical article on "Typhoid Carriers" in Bulletin No. 35 of the Public Health and Marine Hospital Service.

In averaging the results obtained by several observers: Lentz (1905), Klinger (1906), and von Drigalski (1906), he found that of 1,782 cases of typhoid fever, 53, or about 3 per cent., became chronic carriers. The persistence of the typhoid bacilli in the stools is due, at times, he says, "to chronic typhoid ulcerations, but more commonly to the infection of the gall bladder with this bacillus."

12. Soper (1907), Journal of the A. M. A., p. 2019.

REPORT OF THE WEEKLY BACTERIOLOGICAL EXAMINATION OF CROTON WATER FOR THE YEAR 1907.

By W. CAREY NOBLE.

Croton tap water at East Sixteenth street was plated in agar and tested for the presence of colon bacilli once a week during the year. The colony count at 37 degrees and 24 degrees was as follows:

	1 c.c. Plated in Agar.		Quantity of Water Containing Colon Bacilli as shown by the Presumptive Test.
	At 37° C. for 24 hrs.	At 24° C. for 72 hrs.	
January 3.....	70 colonies.	2,780 colonies.	1 c.c.
January 10	105 "	2,900 "	1 c.c.
January 16	53 "	560 "	10 c.c.
January 24	21 "	183 "	10 c.c.
February 8.....	30 "	290 "	10 c.c.
February 14.....	27 "	168 "	10 c.c.
February 20.....	29 "	240 "	10 c.c.
February 28.....	13 "	232 "	1 c.c.
March 8	21 "	320 "	10 c.c.
March 15	43 "	520 "	1 c.c.
March 21	72 "	2,422 "	1/10 c.c.
March 28	48 "	2,290 "	1/10 c.c.
April 4	32 "	109 "	10 c.c.
April 10	57 "	103 "	10 c.c.
April 17	26 "	96 "	10 c.c.
April 24	29 "	102 "	10 c.c.
May 1	13 "	210 "	10 c.c.
May 9	10 "	184 "	10 c.c.
May 15	8 "	83 "	10 c.c.
May 24	18 "	91 "	10 c.c.
May 29	25 "	620 "	10 c.c.
June 5... ..	14 "	710 "	10 c.c.
June 14.....	20 "	490 "	1 c.c.
June 24.....	23 "	310 "	1 c.c.
July 3.....	27 "	107 "	1 c.c.
July 11.....	19 "	162 "	1/10 c.c.
July 17.....	16 "	39 "	10 c.c.
July 25.....	121 "	380 "	1 c.c.

	1 c.c, Plated in Agar.		Quantity of Water containing Colon Bacilli as shown by the Presumptive Test.
	At 37° C. for 24 hrs.	At 24° C. for 72 hrs.	
August 3.....	28 colonies.	210 colonies.	1/10 c.c.
August 9.....	86 "	370 "	1/10 c.c.
August 15.....	11 "	39 "	1/10 c.c.
August 21.....	18 "	30 "	1 c.c.
August 30.....	15 "	70 "	1 c.c.
September 5.....	25 "	37 "	10 c.c.
September 12..... " "	10 c.c.
September 19.....	62 "	185 "	10 c.c.
September 25.....	209 "	420 "	1/10 c.c.
October 2.....	26 "	65 "	.01 c.c.
October 10.....	58 "	71 "	1/10 c.c.
October 19.....	80 "	153 "	1/10 c.c.
October 25.....	52 "	169 "	1/10 c.c.
October 31.....	52 "	211 "	1/10 c.c.
November 7.....	101 "	148 "	1/10 c.c.
November 16.....	64 "	104 "	1 c.c.
November 25.....	92 "	270 "	1/10 c.c.
November 30.....	43 "	115 "	1 c.c.
December 5.....	56 "	163 "	1 c.c.
December 13.....	29 "	80 "	10 c.c.
December 18.....	37 "	103 "	1 c.c.
December 27.....	52 "	142 "	1 c.c.

THE PRODUCTION OF DIPHTHERIA ANTITOXIN DURING THE YEAR 1907.

By DR. EDWIN J. BANZHAF.

The total amount of antidiphtheric citrated plasma produced in the laboratory during the year 1907 was 599,300 c.c. This amount was recovered from 106 bleedings from 16 horses. The average potency was 390 units per c.c. The highest potency was 850 units per c.c., and the lowest 125 units per c.c.

By the process of refining and concentrating the antitoxic citrated plasma, devised in the laboratory,¹ we produced 193 liters of antitoxic globulin solution with an average potency of 1,000 units per c.c. The highest potency was 1,825 units per c.c., and the lowest 700 units per c.c. Also by a process of fractioning the antitoxic citrated plasma, devised in this laboratory,² we produced 62 liters of antitoxic globulin solution with an average potency of 1,250 units per c.c. The highest potency was over 2,000 units per c.c., and the lowest 850 units per c.c.

Horse 262 has produced an enormous quantity of antitoxic serum. This horse entered the antitoxin stable in February, 1904, and soon began to produce a high grade serum. During the year 1904 he produced 81,010 c.c. serum, averaging 496 units per c.c. During the year 1905 he produced 70,085 c.c. serum, averaging 400 units per c.c. During the year 1906 he produced 165,010 c.c., averaging 409 units per c.c. From two bleedings in January, 1907, we recovered 9,800 c.c., with a potency of 450 units per c.c., making the total production from this horse 325,905 c.c., averaging 422 units per c.c.

Under Dr. Parks' direction, horses 318, 319, 320, 322, 323, 324, 325, 326, 327 and 328 were immunized simultaneously against diphtheria and tetanus toxins, each animal subsequently being continued on the toxin to which it responded best. Horses 318 and 320 were the only ones that responded to tetanus toxin, that is to a fairly high potency, whereas horse 320 was refractory to diphtheria toxin.

¹ Gibson, *Journ. of Biol. Chem.*, Vol. 1, p. 161, and Report of this Department for 1905.

² Banzhaf and Gibson, *Journ. of Biol. Chem.*, Vol. iii., p. 253, and Report of this Department for 1906.

After 9 bleedings of horse 318 for tetanus antitoxin, the tetanus immunization was discontinued and the animal was again immunized against diphtheria toxin. After two months' immunization, this horse again produced a fairly high antidiphtheric serum (550 units per c.c.).

The following table shows the production of antitoxin by the individual horses:

Horse Number.	Date of First Injection of Toxin.	Number of Bleedings in 1907.	Citrated Plasma in c.c. produced during 1907.	Highest Potency in Units during 1907.	Average Potency in Units during 1907.
262	2/15/04	2	9,800	450	450
306	2/14/06	2	10,300	300	300
309	9/20/06	2	13,500	200	200
316	10/2/06	2	14,300	525	525
317	11/13/06	1	3,800	200	200
318	1/29/07	19	60,150	600	493
319	1/29/07	13	92,950	600	530
322	2/23/07	5	30,200	625	350
323	3/2/07	22	137,350	850	560
324	3/2/07	2	23,100	125	125
325	3/2/07	9	41,750	300	207
326	3/9/07	5	37,400	600	590
327	3/15/07	2	12,750	400	400
328	3/15/07	1	4,000	550	550
333	6/25/07	10	57,000	400	316
334	7/27/07	9	50,950	500	450

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COLLECTED STUDIES
FROM THE
RESEARCH LABORATORY
DEPARTMENT OF HEALTH
CITY OF NEW YORK

DR. WILLIAM H. PARK, DIRECTOR

VOLUME IV

1908-9

THE J. W. PRATT COMPANY
FIFTY-TWO DUANE STREET
NEW YORK

DEPARTMENT OF HEALTH

OF THE

CITY OF NEW YORK

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Commissioner of Health

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General Medical Officer

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Secretary

my receipt of certificate 1-9-11-98

In the following pages have been collected all the papers published from the Research Laboratory in the laboratory year 1908-9, as well as a number of reports and protocols which were unsuited for publication in the regular technical journals. The recipients of this volume will confer a favor on their colleagues of the Research Laboratory by sending their own publications in exchange. Such pamphlets should be addressed to the Librarian, Research Laboratory, Foot of East Sixteenth Street, New York.

THE EDITOR.

New York, September, 1910.

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THE PERCENTAGE OF CASES OF TUBERCULOSIS DUE TO
THE HUMAN AND BOVINE TYPES OF BACILLI

A Study of the Tubercle Bacilli Cultivated from Four Hundred and
Fifty Consecutive Cases of Human and Animal Tuberculosis.

A PRELIMINARY REPORT*

BY

WM. H. PARK, CHARLES KRUMWIEDE, JR., ELISE L'ESPERANCE, WM.
C. THRO, BERTHA VAN H. ANTHONY, WM. H. WOGLOM,
FREDERICK MONTGOMERY.

Assisted by Louisa P. Blackburn and Angeline Courtney.

The present investigation was begun with the knowledge that investigators had already demonstrated in series of *selected* cases the fact that the bovine type of tubercle bacilli had produced a number of cases of human tuberculosis. This having been determined, it seemed to us that the most promising field for further information was to test a large series of consecutive cases, in both children and adults, *in which no selection was made*. In this way it was hoped that a fair idea of the percentage of human and bovine types of infection, in human tuberculosis, could be determined. One series of cases from the Babies Hospital we consider of special importance because in these very young infants there could be no possibility of a change of type. Another series of cases from St. Mary's Hospital, in which the type of bacilli in a number of chronic glandular cases was determined, is of great value in demonstrating that a persistence of several years in man was not sufficient to alter the characteristics of the bovine type. The results obtained by us refer only to New York City and vicinity, but in a general way they probably apply to other American cities, where the conditions are, at least in many respects, similar.

* Four of the bacteriologists engaged in this investigation were appointed under the authority of the Commission for the Investigation of Acute Respiratory Diseases. To Drs. Hermann M. Biggs and L. Emmett Holt our thanks are especially due for their interest and assistance in carrying on the work. We are also greatly indebted to those who have made this investigation possible by their help in supplying us with the material. The full report will be in the next volume.

The report which is here submitted deals only with the most important tests, but the results obtained have been so uniform that we believe there will be little change when all the experiments have been completed.

Methods of Isolation—No direct cultures were attempted. All material was injected into guinea pigs, which were allowed to live from three to five weeks. At the end of this period the tuberculous organs were removed and thoroughly minced with knife and forceps. This minced tissue was then smeared over the surface of the culture tube. This mincing and rubbing of the material over the culture media we have found preferable to simply cutting the tissue, placing it on the surface of the media and subsequently moving to spread the growth as advised by Theobald Smith. The second opening of the tube, a fruitful source of contamination with moulds, is avoided, and the final growth is equal and greater in amount.

Methods of Cultivation—Egg media of two types have been used for isolation and further cultivation. The ease and simplicity of preparation and the uniformity of the final product make other media for isolation unnecessary.

The egg media were made as follows: Plain egg (Dorset). The whole egg was mixed with 10 per cent. by weight of water and inspissated at 70 degrees C. for two hours in a closed chamber. A few drops of sterile water were finally added to each tube to provide the necessary moisture. Glycerin egg (Lubenau) consisted of ten eggs mixed with 200 c.c. of 5 per cent. glycerin bouillon, 1.5 per cent. acid to phenolphthalein. The eggs were quite fresh. This last point we consider to be a very important detail.

Other media, as glycerin agar, glycerin bouillon and glycerin potato, were also used to determine the cultural characteristics and for further cultivation. The glycerin potato has been the most uniformly successful as a stock medium for further cultivation. The cotton stoppers in each case, with or without dipping in paraffin, were pushed down the tube, which was then closed with a tight fitting charred cork and incubated in the inclined position.

Nearly all the cultures so far isolated have fallen into two distinct groups, eugonic (luxuriant) and dysgonic (sparse). This difference is noticed on plain egg, but to a less marked degree than on glycerin egg. Here the influence of glycerin on the growth of the two types, as noticed by several investigators and carefully elaborated by Cobbett, causes a wide gap in the amount and rapidity of growth of the two types. The dysgonic type commonly fails to grow in glycerin egg from animal tissue, and if transplanted from plain egg to glycerine egg, after the first or second generation, grows but sparsely. The growth consists of thin, flat, spreading colonies 1 to 2 m.m. in diameter, or of a confluent thin, flat, slightly granular, non-pigmented layer, which is usually moist. The eugonic type, on the other hand, grows luxuriantly in most instances on glycerin egg, directly from the animal tissue, and in every case in the second or third generation. The growth is confluent, raised and crumpled or coarsely verrucosa, dry, and in practically every case shows a pink pigmentation in early generations. The pink color depends on certain unknown differences in the different batches of media.

The other media used, as glycerin agar, potato and bouillon, we have found mainly of value as corroboration of the above differences for the following reason. The eugonic strains grow luxuriantly upon these media usually in the second generation and with few exceptions in the third and fourth. The dysgonic strains on the other hand usually fail to grow in the early generations, or if they do the growth is very slight.

The differences in the growth of the two types as given only apply to the first few generations, and to cultures which are three weeks old, the rapidity of growth as well as the amount being an important factor. In later generations and with longer periods of incubation the differences are not so extreme and in a few cultures the gap is closing.

The above description and division into distinct and widely separated types of growth is true of the *great majority* of cultures. There are, however, as would be expected in such closely related varieties of the same species of organism, a smaller number of cultures which tend to bridge the gap between these diverging cultural types. At the pres-

ent time the following classification seems warranted, though further study may make some changes necessary.

The grades of growth here stated refer to the total amount as judged by inspection of the cultures. In each case the best growing tube of any set was considered the typical one. Nine grades were arbitrarily selected: thus Grades 1 to 3 sparse, Grades 4 to 6 moderate and Grades 7 to 9 vigorous. The reason for the selection of glycerin egg has already been spoken of. Besides albuminous media of this type we wished also to select some medium which would contain no coagulable albumins to serve as a more rigorous index of the saprophytism of the two types. After numerous trials potato was found to be the most uniform in its results. In some of the earlier cultures before this routine was established, other media, as glycerin agar and bouillon, were used in the early generations, and in these few instances we shall consider them as falling into one of the groups as though potato had been used, as they seem to be absolutely typical in other respects.

Human Type—Group 1. Glycerin egg, Grade 7 to 8 in the first three generations and pigment on glycerin egg mixtures in the early generations. Glycerin potato, Grade 6-8 in the first four generations.

Group 2. Glycerin egg, same as Group 1. Glycerin potato (a) less than Grade 6, or (b) negative or slight, in first four generations.

Group 3.* Glycerin egg. Grade 4 to 6 with pigment slight or absent in first three generations. Glycerin potato, Grade 3 to 5 in first four generations.

Group 4. Glycerin egg, as in Group 3. Glycerin potato, negative or practically so in first four generations.

Bovine Type—Group 1. Glycerin egg, Grade 0 to 3 for first three generations; usually negative or slight directly from the animal tissue and growth remaining moderate for at least five† generations, viz: not increasing above Grade 6. Glycerin potato, slight or negative in the first four generations.

* No culture of this group is included in the series here reported.

† The limit had been originally set at ten generations, but at the time of correcting proof it was found preferable to change this to five, to throw Group 3 into greater relief. These five generations refer to transfers on glycerine egg, preceding glycerine free media not counted.

Group 2. Glycerin egg, Grade 4 in first three generations. That is, the growth is better than Group 1, but increases slowly in amount in the first five generations. Glycerin potato, slight or very moderate growth in the first four generations.

Group 3. At first, as in Group 1 on both glycerin egg and potato, but rapidly increases in amount of growth in the first five generations, reaching Grade 7, and showing some pigmentation on reaching the higher grades.

When one studies this grouping of the two types there is apparent a gradual gradation from one type to the other. Of the human types no one could mistake Groups 1 and 2 for bovine cultures, nor could Group 1 of the bovine type be mistaken for human cultures, if one had handled even a few cultures on these media. As to Group 3 of the human type, the difference is still sufficiently marked to make a decision as to type on inspection of the tubes of the first few generations. When Group 4 is reached, differentiation is very difficult on these two media. Other media may add some information in these cases, as for instance the ability to grow from glycerin egg on to glycerin bouillon added to the tube, a phenomenon we have not seen in any early bovine culture. Group 3 of the bovine type has been differentiated without exception by the characteristics of the early generations, though when the first culture of this type was encountered we considered the possibility of error. Further observation, however, has shown that the early generations, as we thought, are the diagnostic ones. After a few more generations the distinctions in human Groups 3 and 4 and bovine Group 3 are lost and the last tends to outstrip the other two in vigor of growth. These three groups with Group 2 of the bovine type then tend to bridge the gap and form a complete series of types.

This division into two types according to the cultural characteristics has been uniform with the results of animal inoculations, and in practically every case the rabbit virulence has been correctly predicted from the cultural characteristics.

The following is the method we have employed in testing the virulence on rabbits:

The bacilli are removed from the surface of a subculture which is

less than one month old and the excess of moisture removed by gentle pressure between sterile filter papers before the mass is weighed. The culture medium used for these subcultures, with a few exceptions, has been glycerin egg, and there have been added to the tubes after coagulation of the medium a few drops of glycerin bouillon. The growth immediately above the fluid can be readily emulsified. After weighing, the bacterial mass is emulsified in physiological salt solution, which is added in such amount that 1 c.c. of emulsion represents 1 mg. of organisms. The dysgonic viruses in many instances afforded too little growth for weighing. An emulsion was therefore made and compared with a known emulsion from a eugonic virus, the comparison consisting in the equal ease with which print could be read through each emulsion. A slight amount of saline solution was then added, in order that the error, if any, might fall upon the small side. The intravenous route was decided upon as the most suitable for our purpose, and it has been adhered to throughout the experiment. The dose used has been 1 mg. or .01 mg. With the latter dose the stock suspension was diluted with saline, the amount of the fluid injected being always 1 c.c.

It soon became evident that we had to deal with two varieties of organisms, one of which far exceeds its fellow in virulence. An animal inoculated intravenously with 1 mg. or with .01 mg. of bacilli of the bovine type soon becomes emaciated, the fur loses its lustre, respiration becomes labored and death occurs with the larger dose in from 17 to 38 days, with an average period of 21 days, and with the smaller dose in from 25 to 78 days, with an average of 44 days. Autopsy reveals extreme emaciation and except in rare instances* a generalized tuberculosis involving almost every structure in the body. The lesions in the lymph nodes, and of these particularly the inguinal and the axillary nodes are almost pathognomic. The nodes are enlarged, congested and studded with minute caseous areas. The spleen is three or four times its normal size and riddled with tubercles, and the kidneys are sure to contain on their surfaces an average of from six to ten tubercles each. The liver very commonly contains macroscopic tubercles.

* There were four of these rabbits in which the lesions were localized chiefly in the lungs. The same cultures used in the smaller dose produced generalized tuberculosis.

The lymph nodes of the abdomen are usually enlarged, as are those of the thorax, and commonly show small tubercles which may be caseous. The lungs are always largely consolidated, and are literally a mass of tubercles. Tubercles in the heart muscle are not infrequent. The duration of the illness is shortened by increase of the dose and is also influenced by the individual resistance of the animals. Old rabbits do not seem to be more resistant than young ones.

Separated from such viruses by a wide interval of virulence, are the eugonic viruses. These never cause generalized progressive lesions in doses of .01 mg. or even 1 mg. and a rabbit so inoculated will usually live until carried off by an intercurrent malady. The appearance at autopsy is in striking contrast to the findings described in the preceding paragraph. The animal will be found well provided with subcutaneous and abdominal fat, and the lymph nodes, with few exceptions, are normal in size and appearance. The appearance of the lungs varies with the length of time that has elapsed since inoculation. Six weeks after injection they are pretty extensively involved, for at this point, of course, many of the inoculated organisms are arrested. But that the lesions under discussion are regressive is shown by the fact that many animals have gained weight, as well as by our observation that in rabbits, autopsied after an interval of three or four months, the lungs are pink, well collapsed and with tubercles only sparingly distributed throughout them. Almost always there are a few tubercles in the kidneys. The spleen is of normal size, and it is very rare that a tubercle may be found on its surface. Tubercles in the heart muscle are quite uniformly absent. Occasionally after a long interval there occurs chronic tuberculous-like lesions, such as orchitis, osteomyelitis, adenitis, mastitis or cold abscess.*

As in the cultural classification, so in the virulence as tested on rabbits, the great majority of cultures are included in two widely divergent types. There are, to be sure, some showing intermediate grades of virulence. The viruses which show this variation in virulence have not yet been sufficiently studied to warrant any subdivision of groups as in the cultural characteristics. We are, therefore, not in a position

* Although these lesions were considered tuberculous at first, later observations have thrown doubt on this interpretation.

to say how far there will be a tendency to fill the gap between the two extremes of virulence. We can say, however, that these variations have not given us any reason to doubt the reliability of the virulence test in rabbits as a means of differentiation when the test is properly controlled by repetition where any variation occurs in one or more rabbits of a series.

Wherever any intermediate characteristics of cultures or rabbit virulence have occurred, the combined evidence of both left no doubt as to the type of organism.

In a series of selected cultures we have tested the virulence on young calves. Fifty mgs. of culture emulsified in 5 c.c. of normal saline were injected subcutaneously in the neck. Here again the cultures have fallen into two distinct groups. The human type caused only a local lesion, or at most an extension into the neighboring lymph nodes without producing any effect on the health of the calf. The bovine type, on the other hand, produced an acutely fatal generalized tuberculosis. Varying from these two extremes was the result in one calf receiving a bovine virus, where a chronic and apparently regressive generalized tuberculosis was caused. At the end of this article are given the results in each calf inoculated and the viruses used.

The above description has rather accentuated the variations from the widely separated characteristics of the two types. We have done this to show plainly the fallacy of working with a few cultures and then questioning the existence of two distinct types of tubercle bacilli. This would probably be the result if an investigator should happen upon several variants in a small series of cultures. On the other hand if, as might readily happen, all cultures were absolutely typical of the two extremes his conclusions would be diametrically opposite. In neither case would his conclusions be true. Our own ideas have been modified from time to time, the earlier work pointing to wide divergence of types only, the latter work showing variations from these results. This is really what one would expect from analogy with other bacteria, viz., that in two closely related types of organism there is not a wide gap, but rather two norms around which the two types vary, these variants in no way causing any doubt as to the existence of the two types.

The following tables give the cases investigated with the results of cultural characteristics and rabbit virulence, according to the foregoing descriptions. Wherever there are variations from the extreme types, a detailed description of this virus will be given in the final report of our work, this being impossible at the present time.

The rather arbitrary division of cases under sixteen years from the older cases was adopted, as can be seen from the summary of results, to throw the bovine types mainly into one class and to show clearly the age incidence of these cases.

PULMONARY TUBERCULOSIS—ADULTS

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.*	Diagnosis.
5	Sputum	H. Type 1 †	Non-virulent	A.K.	17 yrs.	F.	1½ yrs.	Pulmonary Tuberculosis
9	"	"	"	J.W.	22	M.	7 mos.	"
10	"	"	"	M.S.	adult	F.	?	"
11	"	"	"	M.S.	16 yrs.	F.	?	"
13	"	"	"	R.R.	adult	?	?	"
22	"	"	"	M.V.	24 yrs.	F.	?	"
26	"	"	"	W.E.	adult	?	?	"
27	"	"	"	A.M.	38 yrs.	?	?	"
32	"	"	"	G.K.	adult	F.	?	"
34	"	"	"	H.W.	adult	M.	2 yrs.	"
35	"	"	"	A.M.	30 yrs.	F.	3 "	"
36	"	"	"	A.S.	adult	?	7-8 mos.	"

- (5) Lesions. "Bronchitis" and subacute pleurisy.
- (9) Lesions. Infiltration of right, lower and upper lobes, beginning consolidation. Prognosis unfavorable.
- (10) No details obtainable.
- (11) Case reported in history as chronic endocarditis. No sputum examination recorded.
- (13) No details obtainable.
- (22) Incipient stage. No further information obtainable.
- (26) "Large lesions in left lung" is the only information that can be elicited.
- (27) No details obtainable.
- (32) No details obtainable.
- (34) Lesions. Involvement of both apices. Prognosis good. Looks perfectly well.
- (35) Lesions. Old lesion in right apex, infiltration of left apex. Prognosis favorable.
- (36) Lesions. Both lungs and pleura, "severe."

* Duration of disease to time specimen was received.

† H. type 1 means Human type, group 1, etc. See explanation, page 10.

PULMONARY TUBERCULOSIS—ADULTS, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
37	Sputum	H. Type 1	Non-virulent	J.B.	19 yrs.	M.	2½ yrs.	Pulmonary Tuberculosis
124	"	"	"	B.P.	adult	?	?	"
38	"	"	"	J.H.	24 yrs.	M.	1½ yrs.	"
39	"	"	"	M.S.	adult	?	?	"
40	"	"	"	A.H.	"	F.	?	"
41	"	"	"	J.W.	"	M.	3 mos.	"
42	"	"	"	D.K.	33 yrs.	M.	2 "	"
43	"	"	"	E.K.	39 "	M.	5 yrs.	"
44	"	"	"	L.T.	40 "	M.	1 yr.	"
46	"	"	"	M.F.	adult	M.	"several mos."	"
47	"	"	"	W.B.	29 yrs.	M.	4 yrs.	"
48	"	"	"	L.L.	35 "	M.	6 mos.	"
49	"	"	"					

- (37) Lesions. Infiltration both apices, both upper lobes involved. Prognosis poor.
- (38) No details obtainable.
- (39) Lesions. Infiltration right apex and left lung. Cervical adenitis. Lymph nodes removed two years ago.
- (40) No details obtainable.
- (41) No details obtainable.
- (42) Lesions. Both apices and upper part of lobes.
- (43) Lesions. Infiltration consolidation and cavity.
- (44) Lesions. Both apices, cavity in left apex? Cervical nodes enlarged. Prognosis bad.
- (46) Lesions. Both apices and part of right upper lobe consolidated. Prognosis fair.
- (47) Lesions. Apices, slight.
- (48) Lesions. Second stage. Apices chiefly affected, no cavities. Prognosis poor.
- (49) Lesions. Consolidation of right upper lobe. On the left side, infiltration from apex down to the third space.

PULMONARY TUBERCULOSIS—ADULTS, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
51	Sputum	H.Type 1	Non-virulent	J.G.	30 yrs.	M.	1 yr.	Pulmonary Tuberculosis
52	"	"	"	L.G.	18 "	M.	2 mos.	"
53	"	"	"	W.Q.	28 "	M.	3 "	"
54	"	"	"	M.K.	42 "	M.	3 "	"
55	"	"	"	J.S.	28 "	M.	6 "	"
56	"	"	"	L.F.	28 "	F.	?	"
57	"	"	"	J.M.	44 "	M.	3 mos.	"
59	"	"	"	?	adult	?	?	"
60	"	"	"	J.D.	45 yrs.	M.	1 yr.	"
64	Adenoids and Tonsils	"	"	T.W.	20 "	M.	8 mos.	"
125	Sputum	"	"	S.T.	19 "	M.	6 wks.	"
75	"	"	"	A.D.	21 "	M.	6 mos.	"
76	"	H.Type 2b	"	M.S.	18 "	M.	4 "	"
77	"	H.Type 1	"					

- (51) Lesions. Right side, apex to third rib involved.
- (52) Lesions. Diffuse infiltration right lung, left apex and upper lobe. Prognosis poor.
- (53) Lesions. Diffuse infiltration left lung and right apex and upper lobe.
- (54) Lesions. Consolidation left apex and upper lobe. Beginning infiltration of right apex.
- (55) Lesions. Disseminated infiltration left lung, and right apex and upper lobe. Cervical nodes enlarged.
- (56) Lesions. Infiltration, right lung.
- (57) Lesions. Infiltration of right apex. Prognosis good.
- (59) Lesions. No details obtainable.
- (60) Lesions. Greater part of right lung consolidated. Infiltration of left upper lobe. Axillary nodes enlarged.
- (64) Lesions. Disseminated infiltration of left lung. Prognosis good.
- (75) Lesions. Infiltration of right apex and upper lobe. Few small areas of infiltration in left lower lobe.
- (76) Lesions. Infiltration of both upper, and upper portions of both lower lobes. General pleural involvement.
- (77) Lesions. "Flatness over lower part of right chest, probably fluid." Prognosis bad.

PULMONARY TUBERCULOSIS—ADULTS, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
78	Sputum	H. Type 1	Non-virulent	J.R.	46 yrs.	M.	?	Pulmonary Tuberculosis
79	"	"	"	J.M.	19 "	F.	4 mos.	"
80	"	"	"	D.F.	37 "	M.	10 "	"
81	"	"	"	C.H.	36 "	M.	6 "	"
82	"	"	"	E.B.	29 "	M.	8 "	"
83	"	"	"	L.B.	42 "	M.	3 yrs.	"
84	"	"	"	J.M.	49 "	M.	6 mos.	"
85	"	"	"	C.B.	34 "	M.	2 yrs.	"
86	"	"	"	M.L.	30 "	M.	6 mos.	"
87	"	"	"	M.Y.	28 "	M.	3 "	"
88	"	"	"	H. S.	29 "	M.	3 "	"
90	"	"	"	A.F.	58 "	M.	7 "	"
92	"	"	"	A.N.	adult	E	6 "	"
101	"	"	"	H.H.	20 yrs.	M.	9 yrs.	"

- (78) Lesions. Extensive infiltration of both lungs. Axillary and cervical nodes enlarged.
- (79) Lesions. Infiltration of right apex and part of upper lobe "cavity."
- (80) Lesions. Infiltration of right apex. Axillary and cervical nodes enlarged. Prognosis fair.
- (81) Lesions. Consolidation both lungs. Prognosis bad.
- (82) Lesions. Infiltration both apices and upper lobes, small portion of lower lobes. Axillary and cervical nodes enlarged. Prognosis doubtful.
- (83) Lesions. Both lungs involved. Prognosis fair.
- (84) Lesions. Infiltration of left apex.
- (85) Lesions. Infiltration of both apices.
- (86) Lesions. Consolidation, right apex and upper lobe. Left apex, infiltrated areas.
- (87) Lesions. Both upper lobes involved.
- (88) Lesions. Consolidation left apex and part of upper lobe.
- (90) Lesions. Consolidation both apices and upper lobes. Prognosis bad.
- (92) Lesions. Infiltration of apices.
- (101) Lesions. Complete infiltration, much consolidation.

PULMONARY TUBERCULOSIS—ADULTS, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
102	Sputum	H. Type 1	Non-virulent	M.G.	29 yrs.	M.	1 yr.	Pulmonary Tuberculosis
103	"	"	"	H.C.	25 "	F.	5 mos.	"
104	"	"	"	P.P.	22 "	M.	2 yrs.	"
105	"	"	"	M.M.	23 "	M.	1 yr.	"
106	"	"	"	L.C.	24 "	F.	6 mos.	"
108	"	"	"	P.Z.	22 "	M.	1 mo.	"
110	"	"	"	D.S.	34 "	M.	1 yr.	"
112	"	"	"	S.J.	23 "	M.	8 mos.	"
113	"	"	"	M.E.	27 "	F.	2 yrs.	"
114	"	"	"	P.R.	45 "	M.	6 mos.	"
117	"	"	"	M.R.	17 "	F.	5 "	"
118	"	"	"	A.W.	35 "	F.	1 yr.	"
119	"	"	"	R.R.	33 "	M.	10 yrs.?	"
120	"	"	"	J.S.	39 "	M.	6 mos.	"
121	"	"	"	C.O.	37 "	M.	2 "	"
124	See 37							

- (102) Lesions. Infiltration upper lobes, and upper part of right lower. Axillary and cervical nodes enlarged.
- (103) Lesions. Consolidation right apex.
- (104) Lesions. Infiltration both upper and upper part of lower lobes. Axillary and cervical nodes enlarged.
- (105) Lesions. Infiltration left apex and upper lobe beginning involvement of right apex.
- (106) Lesions. Consolidation of right lung. Prognosis bad.
- (108) Lesions. Infiltration of left lower and part of upper lobes. Prognosis fair.
- (110) Lesions. Extensive infiltration, both lungs. Axillary and cervical nodes enlarged.
- (112) Lesions. Diffuse infiltration of left lung. Prognosis fair.
- (113) Lesions. Infiltration left upper lobe. Prognosis unfavorable.
- (114) Lesions. Consolidation both lungs with cavities in right upper and left lower lobes. Prognosis bad.
- (117) Lesions. Infiltration, entire lungs. Left apex most marked. Few cervical nodes enlarged.
- (118) Lesions. No details obtainable. Prognosis unfavorable.
- (119) Lesions. Involvement of right apex, upper and middle lobe, also left upper lobe.
- (120) Lesions. Consolidation both apices and upper lobes, areas of infiltration.
- (121) Lesions. Consolidation, right lung and left apex and upper lobe.

PULMONARY TUBERCULOSIS—ADULTS, Cont'd

No.	Material.	Cultures	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis
125	See 64							
126	Sputum	H. Type 1	Non-virulent	J.W.	22 yrs.	M.	3 mos.	Pulmonary Tuberculosis
128	"	"	"	I.W.	42 "	M.	6 "	"
131	"	"	"	V.G.	16 "	F.	2 "	"
133	"	"	"	J.D.	18 "	M.	4 "	"
134	"	"	"	R.H.	27 "	M.	4 "	"
135	"	"	"	D.B.	44 "	F.	2 yrs.	"
136	"	"	"	F.O.	18 "	F.	1 yr.	"
219	"	"	"	P.McC.	23 "	M.	2 yrs.	"
220	"	"	"	J.C.	27 "	M.	14 "	"
221	"	"	"	A.H.	38 "	M.	20 "	"
222	"	"	"	R.L.	30 "	M.	4 "	"
223	"	"	"	W.M.	47 "	M.	3 "	"

- (126) Lesions. Extensive infiltration both lobes. Cavity in left lower lobe. Axillary and cervical nodes enlarged. Prognosis bad.
- (128) Lesions. Both lungs involved. Prognosis fair.
- (131) Lesions. Infiltration and consolidation both apices. Infiltration both upper lobes.
- (133) Lesions. Diffuse infiltration both lungs, marked enlargement of cervical nodes.
- (134) Lesions. Consolidation of right apex and upper lobe. Laryngitis.
- (135) Lesions. Consolidation of right apex, infiltration left upper lobe. Prognosis fair.
- (136) Lesions. Infiltration of right apex and upper lobe. Cavity in upper lobe. Prognosis fair.
- (219) Lesions. Consolidation of right apex and upper lobe, slight involvement of middle lobe, and of left apex posteriorly. Progress acute.
- (220) Lesions. Partial consolidation of right apex and upper lobe with slight involvement of left upper lobe. Progress acute.
- (221) Lesions. Scattered areas of consolidation in both lungs. Progress acute.
- (222) Lesions. Slight involvement of both apices. Generalized bronchitis.
- (223) Lesion. Consolidation of right apex and in upper left lobe. Progress subacute with exacerbation.

PULMONARY TUBERCULOSIS—ADULTS, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
224	Sputum	H. Type 1	Non-virulent	J.B.	54 yrs.	M.	2½ mos.	Pulmonary Tuberculosis
227	"	"	"	F.W.	20 "	M.	4 "	"
228	"	"	"	C.A.	18 "	M.	2 "	"
229	"	"	"	J.L.	32 "	M.	13 "	"
230	"	"	"	S.K.	18 "	M.	2 "	"
231	"	"	"	J.A.	21 "	M.	4 "	"
232	"	"	"	D.M.	36 "	M.	9 "	"
233	"	"	"	F.C.	22 "	M.	1 yr.	"
234	"	"	"	M.D.	24 "	M.	8 mos.	"
235	"	"	"	G.B.	36 "	M.	6 "	"
237	"	"	"	W.R.	51 "	M.	9 "	"
240	"	"	"	J.K.	29 "	M.	8 "	"
241	"	"	"	J.H.	38 "	M.	9 "	"
242	"	"	"	J.B.	49 "	M.	1 yr.	"

- (224) Lesions. Fibroid right lung. Scattered areas in left lung. Progress chronic.
- (227) Lesions. Consolidation of both apices. Progress acute.
- (228) Lesions. Consolidation of left apex and upper lobe. Progress acute.
- (229) Lesions. Consolidation of right apex and upper lobe and of left lower lobe. Progress chronic.
- (230) Lesions. Scattered areas of dullness throughout both lungs. Progress acute.
- (231) Lesions. Scattered areas throughout both lungs. Cavity in left lower lobe. Progress acute.
- (232) Lesions. Both apices consolidated, involvement of left lobe. Progress chronic.
- (233) Lesions. Involvement of both apices and left lower lobe. Progress acute.
- (234) Lesions. Consolidation of right apex and upper lobe.
- (235) Lesions. General involvement. Progress acute.
- (237) Lesion. Fibroid left upper lobe. Infiltration of right upper lobe. Progress chronic.
- (240) Lesions. Right apex and upper lobe consolidated. Progress subacute.
- (241) Lesions. Involvement of both apices and upper lobes. Progress acute.
- (242) Lesions. Involvement of both upper lobes. Progress subacute.

PULMONARY TUBERCULOSIS—ADULTS, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
243	Sputum	H. Type 1	Non-virulent	H.H.	22 yrs.	M.	3 yrs.	Pulmonary Tuberculosis
244	"	"	"	M.K.	29 "	M.	1 mo.	"
245	"	"	"	J.K.	23 "	M.	3 yrs.	"
246	"	"	"	M.S.	50 "	M.	4 wks.	"
248	"	"	"	T.H.	29 "	M.	18 mos.	"
249	"	"	"	D.A.	24 "	M.	6 "	"
250	"	"	"	A.B.	28 "	M.	2½ yrs.	"
251	"	"	"	E.M.	17 "	M.	2 "	"
252	"	"	"	L.E.	26 "	M.	4 mos.	"
253	"	"	"	J.C.	39 "	M.	1 yr.	"
254	"	"	"	J.L.	18 "	M.	16 mos.	"
255	"	"	"	A.R.	50 "	M.	13 "	"
256	"	"	"	E.J.	28 "	M.	2½ yrs.	"
280	"	"	"	M.S.	33 "	F.	5 "	"

- (243) Lesions. Involvement of right apex and upper lobe. Scattered areas, lower lobes. Progress acute.
- (244) Lesions. Left apex and upper lobe, right upper lobe posteriorly consolidated. Progress acute.
- (245) Lesions. Right apex, middle lobe and left base involved. Progress acute.
- (246) Lesions. Right upper lobe and lower lobe posteriorly consolidated. Progress acute.
- (248) Lesions. Consolidation of right apex. Progress acute.
- (249) Lesions. Consolidation of right apex, and upper part of middle lobe. Progress acute.
- (250) Lesions. Right apex and upper lobe and left apex involved. Progress chronic.
- (251) Lesions. Left apex and middle lobe involved.
- (252) Lesions. Consolidation of both apices and right upper lobe. Progress acute.
- (253) Lesions. General dullness. Cervical nodes on right side enlarged. Progress chronic.
- (254) Lesions. Consolidation left apex and upper lobe. Progress chronic.
- (255) Lesions. Consolidation right apex and upper lobe.
- (256) Lesions. Extreme involvement of right lungs. Dullness of left base. Progress chronic.
- (280) Lesions. Infiltration both apices and upper lobes. Prognosis fair.

PULMONARY TUBERCULOSIS—ADULTS, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
285	Sputum	H. Type 1	Non-virulent	S.H.	19 yrs.	M.	4 mos.	Pulmonary Tuberculosis
286	"	"	"	A.R.	34 "	M.	1 mo.	"
287	"	"	"	J.M.	31 "	M.	9 mos.	"
288	"	"	"	W.M.	38 "	M.	8 yrs.	"
289	"	"	"	F.K.	19 "	F.	5 mos.	"
290	"	"	"	G.W.	39 "	M.	2 yrs.	"
294	"	"	"	O.F.	22 "	M.	6 mos.	"
295	"	"	"	H.N.	36 "	F.	1 mo.	"
367	"	"	Not tested	H.G.	30 "	M.	10 mos.	"
296	"	"	Non-virulent	W.	50 "	?	6 "	"
298	"	"	"	B.L.	36 "	F.	8 "	"
300	"	"	"	B.M.	53 "	M.	3 "	"
302	"	"	"	E.B.	37 "	M.	3 yrs.	"
303	"	"	"					

- (285) Lesions. Left apex and upper lobe and upper part of lower lobe involved. Prognosis fair.
- (286) Lesions. Consolidation of right apex and part of upper lobe. Infiltration of left apex. Prognosis fair.
- (287) Lesions. Infiltration right upper lobe. Prognosis poor.
- (288) Lesions. Consolidation right apex and upper lobe, and left apex. Prognosis good.
- (289) Lesions. Disseminated infiltration of left lung. Beginning infiltration of right apex. Prognosis fair.
- (290) Lesions. Infiltration of right apex. Area of consolidation in upper posterior part of left lower lobe. Prognosis fair.
- (294) Lesions. Right apex and upper lobe involved.
- (295) Lesions. Infiltration left apex and upper lobe.
- (296) Lesions. Infiltration both apices.
- (298) Lesions. Consolidation of right apex. Infiltration of right upper lobe and left lower lobe.
- (300) Lesions. Right apex and upper lobe involved.
- (302) Lesions. Right apex involved.
- (303) Lesions. Whole right lung and right apex involved.

PULMONARY TUBERCULOSIS—ADULTS, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
304	Sputum	H. Type 1	Non-virulent	J.C.	41 yrs.	M.	1 yr.	Pulmonary Tuberculosis
305	"	"	"	F.R.	18 "	M.	7 wks.	"
307	"	"	"	E.G.	38 "	M.	1 yr.	"
337	"	"	"	R.R.	16 "	F.	4 mos.	"
338	"	"	"	T. Van N	19 "	F.	6 "	"
339	"	"	"	B.D.	29 "	F.	1 yr.	"
340	"	"	"	M.M.	20 "	F.	1 mo.	"
341	"	"	"	R.I.	32 "	M.	2 yrs.	"
344	"	"	"	H.E.	18 "	M.	2½ "	"
345	"	"	"	P.H.	42 "	M.	13 mos.	"
346	"	"	"	G.G.	35 "	M.	1½ yrs.	"
347	"	"	"	L.C.	24 "	M.	1½ "	"
348	"	"	"	D.L.	46 "	M.	1 yr.	"
349	"	"	"	G.E.	53 "	M.	7 mos.	"
350	"	"	"	E.B.	24 "	M.	4 "	"
351	"	"	"	J.M.	41 "	M.	2¼ yrs.	"
352	"	"	"	B.R.	28 "	M.	2 "	"

- (304) Lesions. Extensive infiltration and softening of left lung.
- (305) Lesions. Right apex and upper lobe involved.
- (307) Lesions. Extensive infiltration of right lung; slight infiltration of left apex. Prognosis poor.
- (337) Lesions. At first reported infiltration right apex; prognosis good. Later reported "no case." Three sputum examinations negative.
- (338) Lesions. Infiltration of both apices. Prognosis good.
- (339) Lesions. Infiltration of right apex and upper lobe and of left apex. Prognosis fair.
- (340) Lesions. Infiltration left lower lobe and slight infiltration of left apex. Prognosis good.
- (341) Lesions. Second stage. Progressive.
- (344) Lesions. Second stage. Bone necrosis of right elbow. Improving.
- (345) Lesions. Second stage. Improving.
- (346) Lesions. Third stage. Progressive.
- (347) Lesions. Third stage. Improving.
- (348) Lesions. Second stage. Progressive.
- (349) Lesions. Third stage. Progressive.
- (350) Lesions. Third stage. Progressive.
- (351) Lesions. Second stage. Progressive.
- (352) Lesions. Third stage. Progressive.

PULMONARY TUBERCULOSIS—ADULTS, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
353	Sputum	H. Type 1	Non-virulent	F.L.	34 yrs.	M.	5 mos.	Pulmonary Tuberculosis
354	"	"	"	H.S.	26 "	M.	6 "	"
355	"	"	"	J.D.	46 "	M.	3½ yrs.	"
356	"	"	"	J.K.	31 "	M.	5½ "	"
357	"	"	"	H.H.	30 "	M.	1 yr.	"
358	"	"	"	A.Z.	30 "	M.	6 mos.	"
359	"	"	"	F.S.	18 "	M.	8 "	"
360	"	"	"	J.L.	43 "	M.	7 yrs.	"
361	"	"	"	C.C.	30 "	M.	7 mos.	"
362	"	"	"	C.C.	21 "	M.	6 "	"
364	"	"	"	P.S.	42 "	M.	1 yr.	"
366	"	"	"	W.J.	30 "	M.	5½ yrs.	"
367	See 295	"	"					
368	Sputum	"	"	B.T.	48 "	M.	8 "	"
370	"	"	"	F.Z.	21 "	M.	6 mos.	"
371	"	"	"	J.M.	31 "	M.	1 yr.	"
373	"	"	"	E.G.	34 "	F.	?	"

(353) Lesions. Third stage. Progressive.

(354) Lesions. Second stage. Improving.

(355) Lesions. Third stage. Progressive.

(356) Lesions. Third stage. Improving.

(357) Lesions. Third stage. Progressive.

(358) Lesions. First stage. Improving.

(359) Lesions. Second stage. Improving.

(360) Lesions. Second stage. Improving.

(361) Lesions. Second stage. Progressive.

(362) Lesions. Second stage. Improving.

(364) Lesions. Second stage. Quiescent.

(366) Lesions. Third stage. Progressive.

(368) Lesions. Third stage. Progressive.

(370) Lesions. Third stage. Progressive.

(371) Lesions. Second stage. Improving.

(373) Lesions. Partial consolidation of both upper lobes, and infiltration of right lower. Prognosis poor.

PULMONARY TUBERCULOSIS—ADULTS, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
374	Sputum	H. Type 1	Non-virulent	P.R.	45 yrs.	M.	2 mos.	Pulmonary Tuberculosis
378	"	"	"	K.S.	39 "	M.	7 "	"
379	"	"	"	J.McG.	26 "	M.	4 "	"
380	"	"	"	F.S.	20 "	M.	11 "	"
384	"	"	"	E.M.	21 "	M.	1 mo.	"
385	"	"	"	E.H.	43 "	M.	3 mos.	"
386	"	"	"	L.W.	30 "	M.	1½ yrs.	"
387	"	"	"	G.C.	18 "	M.	5 mos.	"
390	"	"	"	G.A.	39 "	M.	8 "	"
391	"	"	"	E.H.	51 "	M.	8 wks.	"
398	"	"	"	R.H.	22 "	M.	6 mos.	"
399	"	"	"	B.McK.	73 "	M.	2¾ yrs.	"
400	"	"	"	A.P.	24 "	M.	2 yrs.	"
401	"	"	"	J.McH.	34 "	M.	1¼ yrs.	"
402	"	"	"	M.H.	42 "	M.	1½ "	"
403	"	"	"	M.D.	21 "	M.	7 mos.	"
404	"	"	"	J.C.	29 "	M.	1¼ yrs.	"
405	"	"	"	G.S.	37 "	M.	1 yr.	"
407	"	H. Type 2a	"	H.S.	37 "	M.	2¾ yrs.	"

(374) Lesions. Infiltration left apex and upper lobe. Prognosis fair.

(378) Lesions. Infiltration of both apices. Prognosis fair.

(379) Lesions. Infiltration of right apex, consolidation of left apex. Prognosis poor.

(380) Lesions. Infiltration of right lung and left apex. Prognosis doubtful.

(384) Lesions. Infiltration of right apex. Prognosis fair.

(385) Lesions. Infiltration of left apex and left lower lobe. Prognosis good.

(386) Lesions. Infiltration both apices and upper lobes. Prognosis poor.

(387) Lesions. Infiltration left upper lobe, and slight infiltration of right upper and left lower lobes. Prognosis fair.

(390) Lesions. Infiltration of both apices. Prognosis good.

(391) Lesions. Consolidation of left upper lobe.

(398) Lesions. First stage. Improving.

(399) Lesions. Third stage. Progressive.

(400) Lesions. Second stage. Progressive.

(401) Lesions. Second stage. Improving.

(402) Lesions. Third stage. Progressive.

(403) Lesions. Second stage. Progressive.

(404) Lesions. Second stage. Improving.

(405) Lesions. Second stage. Improving.

(407) Lesions. Second stage. Progressive.

PULMONARY TUBERCULOSIS—ADULTS, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
410	Sputum	H. Type 1	Non-virulent	P.C.	27 yrs.	F.	7 mos.	Pulmonary Tuberculosis
412	"	"	"	W.R.	43 "	F.	10 "	"
413	"	"	"	V.E.	35 "	F.	6 "	"
414	"	"	"	W.H.	40 "	F.	12 yrs.	"
415	"	"	"	H.J.S.	49 "	F.	1 $\frac{2}{3}$ "	"
417	"	"	"	T.L.	47 "	F.	1 yr.	"
418	"	"	"	J.K.	21 "	M.	3 mos.	"
419	"	"	"	N.H.	59 "	M.	3 yrs.	"
420	"	"	"	P.R.	41 "	M.	2 "	"
421	"	H. Type 2b	"	M.S.	44 "	M.	9 "	"
422	"	H. Type 1	"	M.A.	20 "	F.	3 "	"
423	"	"	"	R.L.	21 "	M.	6 mos.	"
425	"	"	"	W.H.	21 "	M.	1 yr.	"
427	"	"	"	F.K.	18 "	M.	4 mos.	"
431	"	"	"	W.B.	45 "	M.	4 "	"
432	"	"	"	E.E.	22 "	M.	1 yr.	"

- (410) Lesions. Second stage. Progressive.
 (412) Lesions. Second stage. Progressive.
 (413) Lesions. Second stage. Progressive.
 (414) Lesions. Third stage. Progressive.
 (415) Lesions. Third stage. Progressive.
 (417) Lesions. Second stage. Improving.
 (418) Lesions. Third stage. Progressive.
 (419) Lesions. Third stage. Progressive.
 (420) Lesions. Second stage. Progressive.
 (421) Lesions. Third stage. Progressive.
 (422) Lesions. Third stage. Progressive.
 (423) Lesions. Second stage. Improving.
 (425) Lesions. Third stage. Progressive.
 (427) Lesions. Left base involved. Prognosis fair.
 (431) Lesions. Infiltration of both apices. Prognosis good.
 (432) Lesions. Consolidation of right upper and middle lobes. Prognosis poor.

PULMONARY TUBERCULOSIS—ADULTS, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
436	Sputum	H. Type 1	Non-virulent	J. McD.	29 yrs.	M.	10 wks.	Pulmonary Tuberculosis
437	"	H. Type 2a	"	S.A.	38 "	M.	7 mos.	"
440	"	H. Type 1	"	W.S.	39 "	M.	11 "	"
444	"	"	"	A.F.	38 "	F.	7 "	"
445	"	"	"	M.M.	37 "	F.	4 "	"
447	"	"	"	M.P.	24 "	?	?	"
448	"	"	"	M. McK.	35 "	F.	3 yrs.	"
449	"	"	"					
588	"	"	Not tested	M.M.	50 "	F.	1½ "	"
593	"	"	"					
450	"	"	Non-virulent	A.C.	44 "	F.	1½ "	"
451	"	"	"	C.B.	34 "	F.	3 mos.	"
452	"	"	"	A.Y.	22 "	F.	1 yr.	"
453	"	"	"	M.P.	42 "	F.	1½ yrs.	"

(436) Lesions. Infiltration of both apices. Prognosis fair.

(437) Lesions. Diffuse consolidation of right lung. Prognosis poor.

(440) Lesions. Infiltration of right upper lobe, left apex and left lower lobe. Prognosis fair.

(444) Lesions. Right lung and left apex involved. Progress acute.

(445) Lesions. Consolidation of right apex. Progress chronic.

(447) Lesions. Slight involvement of right lung. Condition good.

(448) Lesions. Scattered lesions in both lungs. Condition poor.

(449) Lesions. Extensive involvement of both lungs. Progress slow. Condition fair.

(450) Lesions. Involvement of both lungs. Condition fair.

(451) Lesions. Involvement of left lung. Progress slow. Condition good.

(452) Lesions. Involvement of both lungs. Course slow. Condition fair.

(453) Lesions. Involvement of left apex and right lung. Course slow. Condition fair.

PULMONARY TUBERCULOSIS—ADULTS, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
457	Sputum	H.Type 1	Non-virulent	R.M.	32 yrs.	F.	1½ yrs.	Pulmonary Tuberculosis
459	"	"	"	M.P.	60 "	F.	1 yr.	"
460	"	"	"	M.L.	46 "	F.	1½ yrs.	"
461	"	"	"	M.W.	34 "	F.	2¼ "	"
462	"	"	"	G.M.	25 "	F.	1 yr.	"
464	"	"	"	L.M.	36 "	F.	4 mos.	"
465	"	"	"	E.M.	33 "	F.	11 "	"
466	"	"	"	H.J.	45 "	F.	1½ yrs.	"
468	"	"	"	A.M.	59 "	F.	1¼ "	"
469	"	"	"	L.S.	47 "	F.	10 mos.	"
470	"	"	"	M.McN.	36 "	F.	6 "	"
472	"	"	"	A.L.	32 "	F.	3 yrs.	"
473	"	"	"	F.L.	57 "	F.	3 "	"
475	"	"	"	L.D.	adult	F.	3 "	"
476	"	"	"	B.G.	28 yrs.	F.	2 "	"

(457) Lesions. Involvement of both apices. Course slow. Condition fair.

(459) Lesions. Involvement of right upper lobe. Course slow. Condition good.

(460) Lesions. Scattered lesions in both lungs. Condition good. Course slow.

(461) Lesions. Involvement of left lung anteriorly and right base posteriorly. Course slow. Condition fair.

(462) Lesions. Involvement of both lungs. Course slow. Condition fair.

(464) Lesions. Involvement of right lung and left apex. Course slow. Condition good.

(465) Lesions. Involvement of left apex and right lung anteriorly. Course slow. Condition good.

(466) Lesions. Extensive lesions of left lung. Course slow. Condition poor.

(468) Lesions. Scattered lesions in right lung. Consolidation of left lung. Course rapid. Condition, dead.

(469) Lesions. Right upper lobe consolidated. Course slow. Condition good.

(470) Lesions. Left apex involved. Course slow.

(472) Lesions. Involvement of both lungs. Course slow. Condition poor.

(473) Lesions. Involvement of both lungs. Course slow. Condition bad.

(475) Lesions. Consolidation of right apex. Involvement of right lung and left apex. Course slow. Condition good.

(476) Lesions. Left apex consolidated, scattered lesions through left lung. Abscess of cervical nodes, incised four years ago. Condition good.

PULMONARY TUBERCULOSIS—ADULTS

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
477	Sputum	H. Type 1	Non-virulent	A.T.	23 yrs.	F.	?	Pulmonary Tuberculosis
478	"	"	"	M.C.	28 "	F.	1 yr.	"
480	"	H. Type 2a	"	M.C.	39 "	F.	1 "	"
481	"	H. Type 1	"	S.L.	34 "	F.	1 "	"
483	"	"	"	M.L.	88 "	F.	sev. yrs.	"
484	"	"	"	M.H.	30 "	F.	1 yr.	"
485	"	"	"	M.P.	43 "	F.	6 yrs.	"
486	"	"	"	L.M.	29 "	F.	3 "	"
487	"	"	"	L.J.	35 "	F.	5 mos.	"
499	"	"	"	W.A.	35 "	M.	6 "	"
500	"	H. Type 2a	"	?	Adult	?	?	"

- (477) Lesions. Involvement of both upper lobes. Condition poor.
- (478) Lesions. Scattered lesions of left lung. Course slow. Condition good.
- (480) Lesions. Consolidation right lung. Condition good.
- (481) Lesions. Both apices involved. Course slow. Condition poor.
- (483) Lesions. Consolidated right lung. Course slow. Condition poor.
- (484) Lesions. Involvement of right apex and left lung. Course rapid. Condition poor.
- (485) Lesions. Involvement of left apex and right lung. Course slow. Condition poor. Axillary nodes removed five years ago.
- (486) Lesions. Both apices involved. Condition fair.
- (487) Lesions. Consolidation left apex. Course slow. Condition good.
- (499) Lesions. Both apices involved.
- (500) No details obtainable.

PULMONARY TUBERCULOSIS—CHILDREN

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
15	Sputum	H. Type 1	Non-virulent	E.B.	11 mos.	M.	1 mo.	Pulmonary Tuberculosis
50	"	"	"	J.M.	14 yrs.	M.	?	"
156	"	"	"	A.R.	1 yr.	F.	?	"
225	"	"	"	J.F.	15 yrs.	M.	6 mos.	"
277	"	"	"	?	6 "	?	5 "	"
406	"	"	"	E.S.	15 "	F.	11 "	"
409	"	"	"	H.G.	14 "	M.	1 yr.	"
416	"	"	"	J.T.	13 "	M.	1 $\frac{2}{3}$ yrs.	"
471	"	"	"	H.M.	14 "	F.	7 mos.	"
479	"	"	"	J.L.	11 "	F.	2 yrs.	"

(15) History. No tuberculosis in family. Breast fed for four months. Then pasteurized milk (Straus). One month ago the child began to be feverish and to cough, especially in the morning. There was loss of weight.

Physical examination. A rachitic child. Chest expansion poor. Broncho-vesicular breathing over entire upper right lobe, with rales and dullness. Signs of cavity just above the nipple. The left lung shows a few scattered rales. Discharged two weeks later. Condition in lungs unchanged. Child much weaker.

(50) Lesions. Right and lower lobe and left base involved.

(156) History. Nursed for a period of time unstated, and then given cows' milk. Illness began with cough, nasal discharge, otitis media and gastro-intestinal disturbances. The cough increased in severity and the child lost weight. Physical examination, a cachectic child with enlargement of all the superficial lymph nodes. There was consolidation of the right apex, which gradually involved the entire upper lobe, and infiltration of the remainder of the right lung and all of the left. The spleen was palpable. The symptoms gradually increased in severity. Death occurred three months later. No autopsy.

(225) Lesions. Consolidation of entire right upper and part of middle lobe, scattered areas in left upper lobe. Progress acute.

(277) History. Measles about five months ago, followed by a cough, which has persisted until the present. There has been a loss in weight. Involvement of the right apex.

(406) Lesions. Second stage. Progressive.

(409) Lesions. Third stage. Improving.

(416) Lesions. Second stage. Improving.

(471) Lesions. Extensive involvement of left lung. Course rapid. Condition poor.

(479) Lesions. Consolidation of right upper lobe. Lumbar lordosis. Course slow. Condition good.

NOTE.—See Generalized Tuberculosis Children, Nos. 1, 20, 58A, 63, 66, 71, 98, 123, (164 & 165), 273, 279, 292, 388, 392, 435 for other pulmonary cultures.

TUBERCULOUS ADENITIS—ADULTS

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
31	Cervical Nodes	H. Type 1	Non-virulent	S.H.	20 yrs.	M.	?	Tuberculous Adenitis
185	"	"	"	A.J.	33 "	M.	6+ yrs.	"

(31) History. Three years ago lumps appeared in right side of neck, steady increase in size. Physical examination, negative except for enlarged nodes. Excision. Many of the nodes found broken down.

(185) History. Operated on six years ago for cervical adenitis on left side. Soon after the operation the nodes reappeared; two weeks ago he noticed enlargement of those on the right side. Physical examination shows a chain of enlarged nodes in the left sub-maxillary region, extending anteriorly almost to the median line. Several of these are as large as an almond, but most of them are smaller. On the right side, at the angle of the jaw, there is a node the size of a large almond. On both sides there are enlarged nodes in the triangle of the neck as far down as the clavicle. The posterior cervical, axillary and inguinal nodes are all somewhat enlarged. All of the nodes mentioned are freely movable. The heart and lungs are normal.

Operation. Excision. The material examined is a cervical node the size of a filbert. On section this shows a very few white spots; otherwise it is hard and fibrous. The post-operative history was uneventful.

Pathologist's report, tuberculous adenitis.

TUBERCULOUS ADENITIS*—CHILDREN

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease	Diagnosis.
7	Cervical Nodes	H. Type 1	Non-virulent	F.F.	15 yrs.	F.	1 yr.	Tuberculous Adenitis
18	"	H. Type 2a	"	M.deF.	11 "	F.	1 yr. +	"
28	"	H. Type 1	"	C.S.	4½ "	F.	Sev. mos.	"
29	"	"	"	L.D.	12 "	F.	1 wk. ?	"

- (7) History. One year ago a swelling on the left side of the neck was lanced. The sinus healed in about a week, but the swelling remained, and has progressively enlarged. Examination shows on the left side a tonsillar node as large as a lemon and a sub-maxillary node of about the same size. In the posterior triangle the nodes are about the size of a pea. The right sub-parotid node is as large as a walnut, and on this side the sub-maxillary and the posterior cervical nodes are pea size. Excision. Nearly all the nodes were caseous throughout. Completely healed except for a few granulating areas, three months after operation. Pathological examination, caseous tuberculous nodes.
- (18) History. Enlarged nodes were removed from the right side of the neck one year ago, including one below the angle of the jaw the size of an olive, and several in the deep cervical chain. At the time of operation the left sub-parotid was almond size, but it was not removed. One month ago the child had a sore throat, and it was noticed at that time the left cervical nodes were enlarged. Enlargement has been progressive from the time when it was first noticed. Examination shows the left tonsillar node enlarged, with a small abscess superficial and anterior to the sterno-mastoid muscle, the whole mass being about the size of a lemon. The lymph nodes in the posterior triangle are the size of lima beans and the sub-maxillary node is palpable. Excision. All the nodes are caseous. The wound was completely healed twenty-four days afterward. Pathological examination, caseous tuberculous nodes.
- (28) History. A swelling in the neck was noticed several months ago. Examination shows a poorly nourished child with a grossly symmetrical swelling on each side of the neck, most marked beneath the angle of the jaw and over the upper part of the sterno-mastoid. The nodes which make up this tumor are partly matted together and are individually about the size of pigeon eggs. In the posterior triangle the lymph nodes are slightly enlarged and the axillary, inguinal and epitrochlear nodes are palpable. Excision of the right nodes, and one week later of the left nodes. Discharged, cured. Pathological examination, tuberculous nodes, almost completely caseous.
- (29) History. Tonsils removed one year ago. A swelling in the neck was noticed one week ago, following a sore throat. Examination shows in the right sub-maxillary region a mass about the size of a lemon. The nodes in the tonsillar region are palpable and in the posterior triangle they are about the size of peas. The left sub-maxillary node is the size of a pigeon's egg, the tonsillar node of a small olive, and in the posterior triangle there are a few nodes slightly enlarged. Excision of the nodes on the right side. Discharged six weeks later, improved. Pathological examination, tuberculous nodes with very slight caseation.

* Cervical, axillary, inguinal, etc.; those of a thoracic or abdominal cavity given elsewhere.

NOTE.—See Generalized Tuberculosis Children, No. 434 for other cultures from lymph nodes. Also Abdominal Tuberculosis Children, No. 155.

TUBERCULOUS ADENITIS—CHILDREN, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name	Age.	Sex.	Duration Disease.	Diagnosis.
67	Cervical Nodes	H Type 1	Non-virulent	E.H.	6 yrs.	F.	?	Tuberculous Adenitis
94	"	"	"	H.B.	8 "	M.	7 yrs.	"
95	"	"	"	M.Z.	9 "	F.	1 yr.	"
96	"	B Type 1	Virulent	B.B.	21 mos.	F.	8 mos.	"

- (67) History. The lymph nodes on the right side of the neck have been enlarged for some time. The lesion was thought at first to be a simple inflammatory hyperplasia. Examination shows an olive-sized movable node at the angle of the jaw, and a palpable tonsillar node on the left side. Excision. Discharged a month after operation, cured. Pathological examination, many tubercles and a few small patches of caseation.
- (94) History. The child has had nodular swellings on the left side of the neck since infancy. These were operated on nine months ago and the resulting wound healed in three weeks. The swellings have gradually increased in size. Examination shows the left tonsillar node to be about the size of a lemon. The nodes in the posterior triangle are palpable, as are the deep maxillary nodes. Below the angle of the jaw there is a small, superficial abscess. Excision. Discharged, cured, about two weeks and a half after operation. Pathological examination, caseous tuberculous nodes.
- (95) History. The enlarged nodes were first noticed about one year ago, since which time they have been gradually increasing in size. Examination shows on the right side, enlarged nodes behind and below the maxillary angle, about the size of pigeons' eggs. The nodes beneath the sterno-mastoid and in the posterior triangle are palpable. On the left side the cervical, axillary, epitrochlear and inguinal nodes are enlarged. Excision of the nodes on the right side. Discharged six weeks later, cured. Pathological examination, caseous tuberculous nodes.
- (96) History. Best surroundings, no house infection. Received milk of good quality which was not sterilized. About August 20, 1907, enlarged lymph nodes noticed on left side of neck; the temperature at this time varied from 102°-105° F. Has had more or less rise in temperature ever since. About December 15, the nodes on the right side were found to be enlarged. Lymph nodes of left side excised January 2, 1908. They were found to be much broken down and showed many caseous spots. The wound healed by January 15. On April 22 similar nodes excised from right side. Healing was slow and complicated by otitis media and bronchitis, completely healed by summer. No signs remain, the child being in good health.

TUBERCULOUS ADENITIS—CHILDREN, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
116	Cervical Nodes	H. Type 1	Non-virulent	C.F.	2 yrs.	M.	?	Tuberculous Adenitis
149	"	"	"	E.K.	6½ "	F.	1 yr.	"
150	"	"	"	J.C.	10 "	M.	?	"
161	Inguinal Nodes	"	"	G.R.	5 "	M.	5 mos.	"

- (116) History. No history obtainable. Examination shows nodes about the size of an egg on the left side of the neck. The right tonsillar node is somewhat enlarged. Excision. Discharged, cured, six weeks later. A few small palpable nodes found about two months after operation. Pathological report, tuberculous nodes with but little caseation.
- (149) History. One year ago a lymph node in the median line of the neck in the region of the larynx broke down, but soon healed. Two months later a node in the right sub-maxillary region opened spontaneously. Two months ago the left sub-maxillary node became involved. Examination shows large tuberculous ulcers in the sub-mental and sub-maxillary regions. The sub-mental nodes are partly sloughed away. The tonsillar nodes on both sides are enlarged, and the right sub-maxillary and sub-parotid nodes are greatly enlarged and caseous. The nodes in the posterior chain are the size of peas. Excision of nodes both sides of neck. Discharged one month later in good condition with the wound healed, but with a few nodes still palpable. Pathological examination, tuberculous nodes.
- (150) History. Examination shows enlarged lymph nodes about the size of an olive on both sides of the neck. Excision of three nodes from the left side, varying in size from a filbert to a hickory nut. The tonsillar node was also removed. Discharged eight days later, cured. About six weeks later examination showed a few cervical nodes on the left side, and the axillary, epitrochlear and inguinal nodes palpable. Pathological examination, tuberculous nodes with but little caseation.
- (161) History. In January, 1908, the lymph nodes in the left groin became swollen and broke down, leaving a persistent sinus. Excision June 5, 1908, of inguinal nodes the size of a hazel nut, all showing either complete caseation or caseating centers. Discharged three weeks after operation, cured.

TUBERCULOUS ADENITIS—CHILDREN, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
162	Cervical Nodes left side	H Type I	Non-virulent	M.McC.	10 yrs.	F.	2 mos.	Tuberculous Adenitis
201	Cervical Nodes right side	"	"					
163	Cervical Nodes	B Type I	Virulent	F.H.	3 "	M.	3 "	"
167	"	H Type I	Non-virulent	L.S.	4½ "	M.	9 "	"
184	"	B Type I	Virulent	J.T.	4 "	F.	1½ "	"

- (162) History. Enlarged cervical nodes first noticed April, 1908, following a sore throat. Examination, May 26, shows a large bunch of nodes beneath and projecting in front of sterno-mastoid muscle on both sides in tonsillar region, the left showing the larger mass. There are numerous palpable nodes in the left posterior triangles, some large nodes the size of an olive along jugular and beneath angle of jaw, and a few small nodes in lower part of right posterior triangle. Excision of left nodes June 5, 1908, and of right nodes July 7, 1908. Wound in right side did not heal, and on August 31 was open inward as far as trachea and downward as far as mediastinum. Signs of cavity in apex developed; temperature 100°-103. Became gradually worse, and died September 23, 1908. No autopsy.
- (163) History. Three months ago there was noticed a swelling in the neck, which is said not to have increased in size. Examination shows enlargement of the right tonsillar node to the size of a small lemon. It is freely movable. In the sub-maxillary region the nodes are about the size of peanuts, and in the posterior triangle they are enlarged to about the same size. Excision. Discharged, cured.
- (167) History. Swelling in neck of nine months' duration. Examination shows on the left side a swelling in the tonsillar region about the size of a lemon, overlying which there is an area of reddened skin surrounding a small incision. The mass is composed of various-sized nodes, matted together. The posterior cervical and sub-maxillary nodes are palpable. Excision. Discharged, cured, about six weeks after operation. Pathological report, nodes contain miliary tubercles and areas of caseation.
- (184) History. Swelling on the right side of the neck, first noticed early in May, 1908. Examination shows a fluctuating mass about the size of a pigeon's egg, overlying the middle of the jaw. The nodes in the posterior triangle are pea size. Excision June 19, 1908. Discharged about five weeks after operation, a few areas of granulation tissue remaining. Pathological report, chronic inflammation. Not tuberculous.

TUBERCULOUS ADENITIS—CHILDREN, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
201	See 162							
205	Cervical Nodes	H. Type 1	Non-virulent	G.T.	12 yrs.	M.	2½ yrs.	Tuberculous Adenitis
216	"	B. Type 1	Virulent	R.S.	9 "	M.	1½ "	"
217	"	"	"	A.A.	4 "	F.	?	"
247	"	"	"	C.L.	15½ "	F.	5 wks.	"
260	"	"	"	H.H.	15 mos.	M.	1 yr.	"

- (205) History. Father and one brother dead of tuberculosis. Two and a half years ago a swelling in the neck opened spontaneously, and there have been other smaller abscesses since. The present swelling is of six months' duration. Examination shows the right tonsillar node to have attained the size of a hen's egg. The nodes in the anterior chain are olive size, while those in the posterior chain are shot size. On the left side the tonsillar node is the size of a small olive, and the anterior chain is enlarged, but the nodes are of smaller size than the tonsillar. The axillary nodes are pea size and the epitrochlear is palpable. Lymph nodes excised. Discharged twelve days later in good condition with wound healing. Pathologist's report, tuberculous nodes, largely caseous.
- (216) History. Eighteen months ago the lymph nodes on the right side of the neck became enlarged, and seven months ago they began to increase rapidly in size. Examination shows a mass of nodes on the right side, behind the angle of the jaw, each one about the size of a hazel nut. The nodes in the posterior chain are the size of a walnut. Excision. Discharged, cured, seven days after operation. Pathological examination, tuberculous nodes, with many large and small tubercles and some caseation.
- (217) History. Measles recently, since which there has been a persistent rhinitis. Examination shows hypertrophied tonsils, enlarged cervical nodes. Excision of lymph nodes. Discharged about three weeks after operation, cured. Pathological examination, advanced tuberculosis with caseation.
- (247) History. Uncle and aunt died of tuberculosis before the patient was born. The enlarged lymph nodes were first noticed after a cold five weeks ago. Examination shows moderately enlarged nodes at the angle of the jaw on the right side. Excision. Discharged, cured.
- (260) History. About eleven months ago the right side of the neck was lanced; the wound did not heal. Three months afterward another operation was done at the same site. This wound healed, but subsequently reopened. Since the time at which this last operation was done a new swelling on the same side has appeared, and has been gradually increasing in size. Operation. Excision of six or seven nodes in the sub-parotid region, varying in size from a small pea to a small chestnut. These nodes are all firm and readily decapsulated. Post-operative history uneventful.

TUBERCULOUS ADENITIS—CHILDREN, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
262	Cervical Nodes	B. Type I	Virulent	L.O.	9 yrs.	F.	1 yr.	Tuberculous Adenitis
272	Inguinal Nodes	H. Type I	Non-virulent	J.L.	6 "	M.	6 wks.	"
278	Cervical Nodes	"	"	C.C.	10 "	M.	1 yr.	"
375	"	"	"	J.G.	2½ "	M.	6 mos.	"
381	"	B. Type I	Virulent	J.B.	5½ "	M.	10 "	"

(262) History. About a year ago, preceding by five weeks an attack of measles, a small swelling was discovered behind the angle of the jaw on the left side. This remained stationary in size until a month ago, when it began to grow larger. Examination shows, on the left side, a node about the size of a horse-chestnut at the angle of the jaw, and a few small nodes down the side of the neck. On the right side there are numerous enlarged nodes, the largest of which, situated at the angle of the jaw, is about the size of a hickory nut. Excision. Discharged about three weeks after operation cured. Pathological examination, advanced tuberculosis, with large and small tubercles and large areas of caseation.

(272) History. Six weeks ago the child cut his foot on a piece of glass. The wound healed, but opened again, and is now discharging pus. For a week he has had a painful swelling in the groin and some fever. Examination shows an incised wound healed, except for an area in the center. In the groin the lymph nodes vary in size from that of a pea to that of a hickory nut, and there is one node of the latter size just above Poupart's ligament. Excision of ulcer and nodes. Further course uneventful. Pathological examination, tuberculous ulcer. Tuberculous nodes, with many large and small tubercles, but with little caseation.

(278) History. Father died of tuberculosis. One year ago a lump appeared at the angle of the jaw. A month ago this was opened and a little pus escaped. Examination shows nodes varying in size from a pea to a pigeon's egg, beneath the symphysis menti and the border of the jaw. The axillary and inguinal nodes are palpable. Excision. Discharged eleven days after with the wound healed. Pathological examination, tuberculous nodes, largely caseous, with some calcification.

(375) History. Following measles, six months ago, the lymph nodes on the left side of the neck began to enlarge. One of them was incised two weeks after it was first noticed, and a sinus has persisted since that time. Examination shows the right sub-parotid node to be the size of a hickory nut, and enlargement of some of the nodes along the posterior border of the sterno-mastoid. On the left side there is a node of similar size at the angle of the jaw, and a chain extending downward as far as the clavicle. Excision. Discharged cured about three weeks after operation. Pathological examination, many tubercles and small areas of caseation.

(381) History. Sub-parotid nodes in right side of neck enlarged for ten months. Excision. Abscess, sub-maxillary, right side. Caseous nodes have many adhesions about them.

TUBERCULOUS ADENITIS—CHILDREN, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
393	Cervical Nodes	B. Type 1	Virulent	E.C.	4 yrs.	F.	2 yrs.	Tuberculous Adenitis
394	" (right side)	H. Type 1	Non-virulent	M.K.	7 "	F.	2 "	"
395	Cervical Nodes (left side)	"	"					
396	Cervical Nodes	"	"					
442	"	"	"	R.J.	12 "	F.	1 yr.	"

(393) History. There has been a swelling on the right side of the neck for the past two years, which, in the last six months has enlarged rapidly. There are many enlarged nodes on the right side and a subcutaneous abscess just in front of the sterno-mastoid muscle. On the left side there are several nodes at the angle of the jaw which have attained the size of a hazel nut. Excision. Discharged cured about two weeks later. Pathological examination, advanced tuberculosis.

(394) History. Following measles, two years ago, enlarged lymph nodes appeared in the neck. Those on the left side remained quiescent until five days ago, when they began to enlarge rapidly. The nodes on the right side have not enlarged since their discovery, two years ago. Examination shows the right sub-parotid and sub-maxillary nodes enlarged, and an enlarged node on the left side behind the angle of the jaw. Excision of nodes on both sides of the neck. Discharged cured a month afterward. Pathological examination, tuberculous nodes, with large areas of caseation.

(396) History. One and one-half years ago a small lump appeared behind the angle of the jaw. This was removed six months later. Others developed and were removed, and finally another one appeared in the neck and one in the cheek. Examination shows the right sub-parotid to be the size of a hickory nut, and the right sub-maxillary as large as a hazel nut. Both sub-mentals are enlarged. The left sub-maxillary is the size of a hickory nut, and in the left sub-parotid there is a sinus surrounded by keloid scar. Along the border of the sterno-mastoid muscle there is a hard, irregular mass. Excision. Discharged about three and one-half weeks after operation, healed. A few nodes still palpable. Pathological examination, tuberculous nodes, largely caseous.

(442) History. One year ago there was noticed a swelling in the neck. This has steadily increased in size. Examination shows, on the right side, an enlargement of the sub-parotid, sub-maxillary and anterior and posterior chains. These nodes vary in size from that of a pea to that of a hazel nut. The corresponding nodes on the left side are enlarged, but they have not reached the size of those on the right. In the groin there are a few nodes palpable. Excision of the nodes on the right side. Discharged two weeks after operation, healed.

GENERALIZED TUBERCULOSIS. ADULTS

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
308	Spleen	H.Type 1	Non-virulent.	S.E.	23 yrs.	M.	4 mos.	Generalized Tuberculosis

(308) History. Family history is negative, and he has always been well and strong. On June 26, 1908, he had a chill followed by sweating; this recurred every night until very recently, when they became somewhat irregular in appearance, sometimes skipping a night. There has been a slight headache present most of the time, but no vomiting or joint pains. The night before admission, July 14, 1908, he was seized with a sharp shooting pain in the right side, in the area between the sixth and tenth ribs. This pain was relieved upon sitting up; breathing had no effect on it, and it was not made worse by coughing. In the right abdomen there has been present a very severe pain, which is aggravated by breathing and by coughing. The leucocyte count on admission is low, the differential count normal, and a blood culture is sterile. After admission there was a gradual decline in strength, the sputum became blood tinged, larger hæmoptyses appeared and death occurred October 27, 1908.

Autopsy. Anatomical diagnosis, general miliary tuberculosis involving liver, lungs, spleen, kidneys, peritoneum, intestine, lymph nodes and pleura.

Peritoneum smooth and glistening. The omentum is rolled into a thick shotty cord along the right border, and contains small white areas throughout. On the visceral peritoneum there are occasional conglomerated areas. The spleen is surrounded by adhesions. The right lung is more or less adherent to the diaphragm. Heart normal. Left lung almost entirely solidified with caseation of the lymph nodes at the hilum and a cavity in this region communicating with the large bronchi. The right lung contains small shotty areas throughout, and its lymph nodes are enlarged. The peritracheal nodes are large and cheesy. Stomach, duodenum and jejunum normal. The ileum contains two ulcers about the size of a five-cent piece, and occasional miliary tubercles in its mucosa. Colon normal. Liver surface dotted with numerous small white areas. At the hilum of the gall bladder there are a number of enlarged nodes. Spleen—a large number of enlarged caseous areas, some of which are conglomerate and all of which are cheesy in the center. Pancreas normal. Left kidney, small white areas in the cortex. Right kidney same as left.

GENERALIZED TUBERCULOSIS—ADULTS, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
336	Spleen	H. Type 1	Non-virulent	H.G.	29 yrs.	M.	1 yr.	Generalized Tuberculosis

(336) History. Previous history, negative except for smallpox in early youth. He has been in poor health for a year and unable to work for the past six months. During the year he has had a slight cough, with night sweats and loss of flesh. He has had also pain in the abdomen and has been constipated. Died.

Autopsy. Anatomical diagnosis, tuberculous enteritis and peritonitis, septic peritonitis, tuberculosis of lungs, liver, spleen, kidneys and pleura. The peritoneum is covered with small tubercles and the intestinal coils are glued together. There are about 150 c.c. of yellowish-green, foul fluid in the peritoneal cavity. The omentum is rolled up on the transverse colon and is very much thickened, sections of it showing large caseous areas. In the mesentery of the ileum and of the ascending colon there are large caseous masses. Heart negative. Left lung firmly adherent to pleura, diaphragm, pericardium and mediastinum. It is firm and slightly crepitant and shining through the pleura are small white areas. On section, the lung is seen to be studded with many small tubercles. Right lung, adherent to pleura on all sides, with the upper lobe, which contains an enormous number of small tubercles, practically consolidated. Peribronchial nodes not caseous. Stomach normal except for a few tubercles along the lesser curvature on the anterior surface. Pancreas, duodenum and jejunum normal. In the ileum there are ragged, shelled-out ulcers extending through the peritoneum, transverse in their long diameter. The cæcum is thickened and there is a large ulcer in the mucosa. Liver, tubercles on its surface and in its substance. Spleen, many tubercles on the surface, as well as in the substance. Both kidneys contain a moderate number of tubercles on their surfaces.

GENERALIZED TUBERCULOSIS—CHILDREN*

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
1	Sputum	H. Type 2a	Non virulent	A.R.	5 mos.	M.	2½ mos.	G Miliary Tuberculosis
45	Feces	"	"					
16	Mesen- teric Node	H. Type 1	"					
19	Spleen	"	"	J.C.	1½ yrs.	M.	?	Generalized Tuberculosis including Meninges
20	Bronchial Node	"	"					
45	See No. 1							

- (1) History. Mother died of tuberculosis. Breast fed at first, later Nestle's food. Illness began 2½ months ago, January 7, 1908, with cough. Gradual emaciation and temperature fluctuating around 98° and 99°, occasionally rising to 101°-102°. Died March 20, 1908.

Autopsy. Bronchial nodes enlarged on right side. One of these is softened. On left side these nodes contain cheesy tubercles, but are scarcely enlarged. Mediastinal nodes enlarged. Upper lobe of right lung has pleural adhesions over its posterior surface. These tear when the lung is removed and reveal a cavity 1½x1½ inches, containing greenish pus and cheesy material, and communicating with the main bronchus. What is left of the apex is in a condition of cheesy pneumonia, and there are many cheesy tubercles on the surface of the lobe. The anterior half of the upper lobe and the entire middle lobe are well aerated and studded with gray and yellow tubercles. The left apex contains an area of cheesy pneumonia anteriorly. There are many tubercles throughout the substance of both lobes. Heart normal. Spleen contains many very small tubercles. The liver is congested, and contains a few early tubercles. The colon shows about six small round tuberculous ulcers not involving the peritoneum. The solitary follicles of the small intestine and Peyer's patches are ulcerated, some of the ulcers being longitudinal and others transverse. There are a few young ulcers in the duodenum and many in the ileum which involve the peritoneal coat. The kidneys are congested and the capsule free. The left kidney contains one tubercle. The brain contains no tubercles. The pia is oedematous.

- (16) History. Foundling child. Given out to board. Three other children given to the same woman died of tuberculosis. She had an adult boarder with tuberculosis who helped care for the children. Feeding consisted of condensed milk, oatmeal and milk. Details as to clinical course of disease not obtainable. Cause of death given as marasmus.

Autopsy. Anatomical diagnosis, tuberculosis of brain, lungs and liver, spleen and lymph nodes. Cervical nodes tuberculous. Lungs tuberculous. Mediastinal and bronchial nodes caseous. Liver, many tubercles. Spleen, many tubercles. Kidneys, negative. Small intestine, small tuberculous nodules at intervals. Large intestine, normal. Mesenteric nodes enlarged and caseous. Brain, increase of fluid and a few miliary tubercles along the vessels of the temporal lobes.

* Case No. 382 is a primary intestinal tuberculosis. No. 122 is probably of the same type. These two cases follow in this series (see pages 52 and 47).

GENERALIZED TUBERCULOSIS—CHILDREN, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
58 A	Sputum	H. Type 1	Non-virulent	J.C.	5 mos.	M.	3 wks.	Generalized Tuberculosis Including Meninges
58 B.	Cerebro-spinal Fluid	"	"					
62	Mesenteric node	B. Type 1	Virulent	M.B.	3½ mos.	F.	?	Generalized Tuberculosis

(58A) History. Breast fed. Illness began February 18, 1908, with irritability, fever and vomiting. In a few days convulsions occurred. The head was retracted, respiration was irregular and the child was drowsy. The pupils were widely dilated and did not react to light, and there was marked internal strabismus. Kernig's sign was prominent. Lumbar puncture resulted in the withdrawal of 20 c.c. of clear fluid, in which tubercle bacilli could be demonstrated. Died March 8, 1909.

Autopsy. Anatomical diagnosis, acute generalized miliary tuberculosis, involving lungs, liver, spleen, lymph nodes, intestines and kidneys. Lungs, no pleurisy. Miliary tubercles studded over lobes of both lungs. Pea-sized cheesy nodule in right lower lobe posteriorly. Bronchial nodes, largest on right side, where one measures 1 inch in length and is softened. On the left side these nodes contain miliary tubercles. Heart, normal. Spleen, 4x2x2 inches, and closely studded with tubercles. Liver, miliary tubercles throughout. Stomach, normal. Kidneys, congested. Capsule, free. A few tubercles in cortex. Intestines, throughout duodenum and jejunum all the solitary follicles are enlarged and ulcerated; but in no case is the peritoneal coat involved. No ulcers in ileum or colon. Examination of brain not permitted.

(62) History. Fed on cow's milk. Entered hospital February 3, 1908, for repair of harelip and cleft palate. Physical examination was negative. On February 4, 1908, was given a routine Calmette test, which was positive. The physical examination was repeated, with negative findings as before, but tubercle bacilli were found in the sputum. After the operation the temperature rose and cough appeared, with rales in the lungs. In a few days consolidation appeared in the right upper lobe posteriorly, child grew steadily worse and death took place March 18, 1909.

Autopsy. Anatomical diagnosis, acute miliary tuberculosis of pleura, lungs, spleen, liver, intestine, lymph nodes and kidney, broncho-pneumonia. Lungs, no pleural adhesions. Tubercles along parietal pleura on both sides. Right lung, upper lobe completely solid from a recent broncho-pneumonia and a widespread involvement with tubercles. Two cheesy nodules, one near the center and one near the posterior border, each ¼ inch in diameter. Middle lobe well aerated and studded with tubercles. The lower lobe is broncho-pneumonic along the posterior border and closely studded with tubercles. Left lung, apex contains a small area of pneumonia, and tubercles are very numerous. The lower lobe contains an area of pneumonia at the posterior superior angle. The tubercles are smaller and less numerous than in the upper lobe. Bronchial nodes on the right side, the largest one is cheesy and softened, while the others contain only recent tubercles. The left are scarcely enlarged and do not show tubercles. The cellular tissue in mediastinum and the aorta contains numerous tubercles. Stomach normal. Duodenum normal. Jejunum contains three early ulcers in the solitary follicles. Ileum, one early ulcer in a Peyer's patch. Colon, normal. Mesenteric lymph nodes, slightly enlarged. Many of them contain miliary tubercles. Spleen, 3x1½ inches, with many gray tubercles on the surface and in the substance. Peritoneum, normal. Kidneys, congested, with numerous tubercles on surface and cortex.

GENERALIZED TUBERCULOSIS—CHILDREN, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
63 69	Lung Mesenteric Nodes	B. Type 1 "	Virulent "	E.H.	1¼ yrs.	F.	?	Generalized Tuberculosis
66 71	Bronchial "	" H. Type 1	" Non-virulent					
				M.I. W.G.	2⅔ " 7½ mos.	F. M.	? 2 wks.	" " including Meninges

- (63) History. No details obtainable. Foundling child given out to board. Milk fed.

Autopsy. Anatomical diagnosis, empyema, tuberculosis of lungs, bronchial nodes, mesenteric nodes and intestines. Pleura, right, large empyema involving entire cavity; left, negative. Lungs, right, masses of breaking-down caseating pneumonia in both lobes; left, small, scattered tubercles. Bronchial nodes enlarged and caseous. Heart, liver and kidneys, negative. Spleen, many tubercles. Small intestine, several tuberculous ulcers. Large intestine, negative. Mesenteric nodes, enlarged and cheesy.

- (66) History. Foundling child. Given out to board. Died of scarlet fever and measles. No further details obtainable.

Autopsy. Anatomical diagnosis, tuberculosis of cervical nodes, broncho-pneumonia, fatty liver, tuberculosis of bronchial and mesenteric nodes, ulcerative laryngitis. Tongue and larynx ulcerated. Cervical nodes, much enlarged. Lungs, pleura normal. Posterior half of both lobes consolidated. Right middle lobe anteriorly studded with miliary tubercles. Mediastinal nodes enlarged. One very large caseous node at bifurcation of trachea. Heart, negative. Liver, fatty. Kidneys, negative. Small intestine, Peyer's patches congested. Large intestine, coat thickened and congested. Mesenteric nodes enlarged and cheesy.

- (71) History. Breast fed. Illness began May 9, 1908, with vomiting. The child soon became prostrated and feverish, and convulsions occurred. There was no cough. The child grew stuporous, Kernig's sign was positive and rigidity of the extremities and of the neck appeared, without retraction of the latter. Lumbar puncture resulted in the withdrawal of 40 c.c. of clear fluid, in which tubercle bacilli were found. The coma gradually deepened and death supervened May 22, 1908.

Autopsy. Anatomical diagnosis, tubercular meningitis, acute miliary tuberculosis of lungs, liver, spleen and lymph nodes. Brain, tubercles are very numerous along the blood vessels of the internal and upper surfaces of both hemispheres. The tubercles are very small and recent. At the base the pia in the interpeduncular space is very cloudy and is thickened and studded with many tubercles. Tubercles are numerous over the upper surface of the cerebellum. The lateral ventricles are not dilated. Heart, normal. Lungs, no pleurisy, pneumonia at the right apex. There are a few recent tubercles in all the lobes, but no cheesy nodules are present. Bronchial lymph nodes, on the right side two are 1 inch in diameter and show central softening; on the left side they are not enlarged, nor do the mediastinal nodes show any enlargement. Spleen, 3x1½x1 inches. It is closely studded with miliary tubercles, a few of which are yellow and cheesy. Liver, tubercles scattered throughout its substance. It is not enlarged. Stomach, normal. Intestines, no ulcers. Peyer's patches, as well as the solitary follicles, are slightly enlarged. Mesenteric nodes somewhat enlarged, but without tubercles. Kidneys, pancreas and peritoneum, normal.

GENERALIZED TUBERCULOSIS—CHILDREN, Cont'd

No.	Material	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
74	Cerebro-spinal Fluid	H. Type 1	Non-virulent	M. Z.	19 mos.	F.	1 mo.	Generalized Tuberculosis Including Meninges
97	Liver	"	"	M. McD.	6 mos.	F.	?	Generalized Tuberculosis
98	Lung	"	"					
99	Spleen	"	"					
100	Mesenteric Nodes	"	"					

(74) History. Breast fed 17 months, then table food. Measles a month ago, followed by a cough, which has persisted until the present time (April 4, 1908). For four days has been vomiting. Is prostrated and moans constantly. Lymph nodes palpable in the neck and groin. Internal strabismus and rigidity of the neck soon developed, and the child became gradually unconscious, screaming, however, if disturbed. Lumbar puncture resulted in the withdrawal of 40 c.c. of fluid, in which tubercle bacilli were found. Died April 14, 1908.

Autopsy. Anatomical diagnosis, general miliary tuberculosis, involving the brain, pericardium, pleura, omentum, lymph nodes, lungs, liver, spleen, pancreas, intestines, kidneys and meninges. Bronchial nodes, one on the right side is 1 inch long and contains cheesy tubercles. On the left side many are cheesy but not soft. None are larger than $\frac{3}{8}$ inch. Lungs, no adhesions. Miliary tubercles on both visceral and parietal layers of pleura; left, a cheesy area $\frac{3}{4} \times \frac{3}{4}$ inch in apex. Strip of atelectasis along posterior border of lower lobe. Miliary tubercles in large numbers. Right, miliary tubercles in large numbers; no consolidation. Mediastinal nodes, all enlarged and cheesy. Pericardium shows a crop of miliary tubercles close to a tubercular mediastinal node. Miliary tubercles around origin of pulmonary artery, aorta and base of pericardial sac. Stomach, normal. Intestine, some cheesy solitary nodules throughout jejunum and ileum. One Peyer's patch just above the ileocaecal valve contains an early tuberculous ulcer. Mesenteric lymph nodes, slightly enlarged, containing cheesy tubercles. Pancreas, small group of cheesy tubercles in head. Spleen, $3\frac{1}{2} \times 2 \times 1$ inches. Very closely studded with miliary tubercles, many of which are cheesy and measure 1-16 inch in diameter. Liver, closely studded with miliary tubercles. Omentum, studded with miliary tubercles. Parietal peritoneum, no tubercles. One ounce of clear fluid in the peritoneal cavity. Kidneys, 6 to 10 tubercles in the cortex. Brain, many miliary tubercles along the vessels over the cortex of both hemispheres. At the base there is an exudation of fibrin and pus. The ventricles are distended.

(97) History. No details obtainable.
Autopsy. No details obtainable.

GENERALIZED TUBERCULOSIS—CHILDREN, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
122	Mesenteric Node	B.Type 1	Virulent	F.A.	3½ yrs.	M.	?	Generalized Tuberculosis
123	Lung	"	"					
148	Cerebro-spinal Fluid	H.Type 1	Non-virulent	J.VanH.	1½ "	M.	3 wks.	Generalized Tuberculosis Including Meninges

(122) Foundling child, boarded out to different nurses. No definite history of feeding. As far as ascertainable received bottled or store milk which was scalded, according to two of the nurses. At six months of age was in poor health, but later improved till final illness. Measles and broncho-pneumonia, with purulent otitis media, terminating in death. Duration two months.

Autopsy. Anatomical diagnosis, broncho-pneumonia, tuberculosis of mesenteric nodes. (No statement as to whether broncho-pneumonia was tuberculous or not.)

(148) History. Breast fed until one month ago, then table food. Illness began three weeks ago with projectile vomiting. After three days fever appeared, with pain on moving. The child was drowsy but conscious. Later rigidity of extremities and neck appeared, followed by convulsions. The pupils were dilated and reacted sluggishly. Axillary and inguinal nodes just palpable. Lumbar puncture resulted in the withdrawal of 90 c.c. of clear fluid in which tubercle bacilli were found. Died May 28, 1909.

Autopsy. Anatomical diagnosis, acute miliary tuberculosis, involving lungs, liver, spleen and lymph nodes, broncho-pneumonia, fatty liver. Heart, normal. Lungs, no pleurisy. Small wedge-shaped area of tuberculosis in right apex, as well as in the posterior portion of the middle lobe. Recent miliary tubercles over and throughout all the lobes, but most numerous in the right middle lobe. Bronchial nodes, on right side, two nodes, 1 inch long, cheesy and softened; on the left side the nodes are small and red. Anterior mediastinal nodes slightly enlarged and contain tubercles. Stomach, normal. Liver, moderately fatty, with many tubercles throughout its substance. Spleen, 3 inches long, firm and studded with miliary tubercles, several of which are cheesy. Kidneys, congested. Intestine, solitary follicles enlarged, especially in the cecum. The mucosa of the colon is congested. The ileum has a congested mucosa, with enlarged solitary follicles, but no ulcers. Mesenteric nodes, small. A few contain cheesy tubercles. Brain, not examined.

GENERALIZED TUBERCULOSIS—CHILDREN, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
164	Lung	H. Type 1	Non-virulent	I.B.	5½ yrs.	F.	1½ yrs.	Generalized Tuberculosis
165	Bronchial Node	"	"					
273	Lung	"	"	J.K.	1 yr.	M.	7 wks.	"
274	Spleen	"	"					

(164) History. Operated on one and one-half years ago for cervical adenitis. Sinus still present. Measles two months ago, complicated by broncho-pneumonia. Ankylosis of right shoulder joint.

Autopsy. Anatomical diagnosis, unresolved broncho-pneumonia; pulmonary tuberculosis; tuberculosis of bronchial and mesenteric nodes; tuberculous ulceration of intestines.

Cervical nodes, enlarged and cheesy; sinus present. Pleura thickened and adherent. Lungs—Right, broncho-pneumonic consolidation, cheesy, with pus exuding from bronchioles, cavity size of walnut in apex. Left, consolidation in both upper and lower lobes. Mediastinal and bronchial nodes enlarged, cheesy and tuberculous. Heart, liver, kidneys, spleen normal. Small intestine, congestion in patches. Large intestine, small ulcerations, apparently tuberculous. Mesenteric nodes enlarged and tuberculous. Abscess of left hip, ankylosis of right shoulder, both tuberculous in origin.

(273) History. Breast fed until one week ago. Five weeks ago, about August 9, 1908, diarrhoea began and later vomiting appeared. Gastro-enteritis continued and child died September 30, 1908.

Autopsy. Anatomical diagnosis, tuberculosis of bronchial, tracheal and cervical lymph nodes, miliary tuberculosis of lungs and spleen, gastro-enteritis, parenchymatous nephritis, undescended testicle, fatty degeneration of heart, liver and kidneys. Peritoneum, normal. Mesenteric nodes, enlarged and show on section, medullary swelling. Pleura and pericardium, normal. Heart, fatty degeneration. Lungs, do not collapse. Numerous areas of interstitial emphysema. On section they are anemic and markedly edematous. Pleura contains a moderate number of miliary tubercles. Miliary tubercles found upon sectioning the lung. Bronchial nodes, right, normal; left, extensive tuberculous involvement. The lymph nodes at the bifurcation of the trachea are also extensively involved. In the neighborhood of these nodes the lungs contain numerous miliary tubercles. Lower cervical nodes also involved. Spleen, enlarged and congested, with many miliary tubercles throughout its substance. Kidneys, capsule strips easily. Cortex swollen and marking obliterated. Pyramids slightly congested. Liver, fatty. No tubercles. Stomach, normal. Lower part of ileum and the large intestine contain altered blood. Mucosa of entire gastro-intestinal tract thickened and congested. Ileum and large intestine show numerous small hemorrhages and superficial erosions. Peyer's patches, slightly swollen. No evidences of tuberculosis. Pancreas normal. Brain could not be examined.

Histological examination. Kidney, extensive fatty degeneration. No tubercles. Liver, extensive fatty degeneration and infiltration. No tubercles. Spleen, moderate number of epithelioid tubercles. Mesenteric nodes, inflammatory hyperplasia. No tuberculosis.

GENERALIZED TUBERCULOSIS—CHILDREN, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
276	Mesenteric Nodes	H. Type 1	Non-virulent	C.S.	14 mos.	M.	2 wks.	Generalized Tuberculosis

(276) History. Heated milk, then house diet from eight months old. No breast feeding. Two weeks ago, September 22, 1908, began to cough and to be feverish. In November adult in family found to have bad pulmonary tuberculosis. Temperature fluctuated from 99° to 104°. Signs in lungs grew gradually more marked, the child grew progressively weaker and finally died October 6, 1908.

Autopsy. Anatomical diagnosis, general tuberculosis, involving pleura, lungs, lymph nodes, spleen, liver, peritoneum and intestine. Peritoneum, contains about an ounce of dark, bile-stained fluid. Parietal peritoneum in region of liver shows a number of cheesy tubercles, from pin-point size to $\frac{1}{8}$ inch in diameter. The omentum is closely studded with miliary tubercles. The lower surface of the diaphragm is adherent to the liver, and contains many yellow tubercles. Pleura, adhesions on right side, but not on left. Visceral and parietal pleura contain many tubercles. Lungs—Left, an exudate of recent thin fibrino-pus is found between the lobes and extends over the surface of the upper lobe, tubercles over the entire lung. Apex solid with broncho-pneumonia, as is also more than half of the lower lobe. Right, lower lobe contains scattered areas of broncho-pneumonia. The middle lobe is well aerated, and closely studded with miliary tubercles. The upper lobe shows an apex solid from broncho-pneumonia, while the anterior portion of the lung contains a cheesy mass 2x2 inches in diameter on the surface, and within a cavity $1\frac{1}{2}$ x2 inches in dimension. The cavity contains thin grumous material, and communicates with a large bronchus. Bronchial lymph nodes, very much enlarged. The largest on the right side is 1 inch in length and cheesy. None are softened. The mediastinal lymph nodes are all enlarged. At the level of the right clavicle, two lymph nodes are cheesy. Cervical nodes, slightly enlarged; no tubercles. Heart and stomach, normal. Spleen, much enlarged, with miliary tubercles on the surface and several cheesy tubercles in the substance. Liver, enlarged and studded with miliary tubercles, several of which are cheesy. There is a cheesy lymph node in the long fissure. Intestines, no ulcers in colon, but several in the ileum, just above the valve. These, of which there are about six, including a few in the jejunum, extend to the peritoneal coat. Mesenteric nodes, all enlarged and most of them cheesy. Pancreas, normal. Kidneys, soft and red, with the capsule free. There is a large number of tubercles in the capsule and in the cortex, several large cheesy areas in the medulla. Retroperitoneal nodes, cheesy.

GENERALIZED TUBEROULOSIS—CHILDREN, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
279	Bronchial Nodes	H. Type 1	Non-virulent	J.F.	1 yr.	M.	2 mos.	Generalized Tuberculosis
291	Mesenteric Nodes	"	"	C.F.	8 mos.	M.	?	"
292	Bronchial Nodes	"	"					

(279) History. Mother has pulmonary tuberculosis. Breast fed for eight months. Six weeks ago, August 1, 1908, began to cough, and since then has coughed continually. Sleeps well. Temperature range while in hospital, from 98° in the morning to 101° in the evening. Died October 4, 1908.

Autopsy. Anatomical diagnosis, tuberculosis of lungs, lymph nodes, spleen, liver, pleura, peritoneum, kidney and intestine. Lungs, no pleural adhesions except between right middle and upper lobes, where they are fairly well organized. Both lungs closely studded with gray miliary tubercles. The right middle lobe, which is the only consolidated portion, is completely solidified, and shows in its central portion a cavity 1 inch long and $\frac{1}{4}$ inch wide, containing red grumous material, and showing yellow tubercles in its walls. The blood vessels in the wall are much congested, so that the whole cavity has a red instead of a yellow color. There are very small scattered areas of pneumonia in the right upper lobe, and many of the tubercles in the substance of the lobe are conglomerated and cheesy. Congestion is very marked. Bronchial lymph nodes, the largest on the right side measures 1 inch in length and is completely cheesy. On the left side they are small and contain only tubercles. Mediastinal and deep cervical lymph nodes, all of these are enlarged, and some are cheesy. Trachea and larynx, congested. Pericardium, normal. Heart, normal. Foramen ovale closed. Thymus, normal. Spleen, slightly enlarged, firm and closely studded with gray tubercles. Liver, deeply congested, showing gray tubercles on the surface and around the bile ducts on section. Diaphragm, on the right side there are many tubercles, on both pleural and peritoneal surfaces. The remainder of the peritoneum is normal. Pancreas and suprarenals, normal. Kidneys, congested. Tubercles in boundary zone. Left kidney contains a yellow, adherent mass in the pelvis. Stomach normal. Duodenum, normal. Jejunum, several small round tuberculous ulcers, not involving the peritoneum. Ileum, about eight tuberculous ulcers in the solitary follicles outside of Peyer's patches. Cecum, a number of small round ulcers which are continued into the colon, and even into the rectum. In no case do they extend into the peritoneum. The mucosa between the ulcers is congested and the solitary follicles are enlarged. Mesenteric lymph nodes, all enlarged. The majority of them contain cheesy tuberculous areas, but no one of them is completely cheesy.

(291) No history obtainable, except that the child died of generalized tuberculosis. Foundling child, given out to board.

GENERALIZED TUBERCULOSIS—CHILDREN, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
377	Mesenteric Nodes	H. Type 4	Non-virulent	L.P.	3 mos.	M.	?	Generalized Tuberculosis

(377) History. Mother died of tuberculosis when baby was ten days old. Fed on cows' milk in various modifications. Appetite never good. On October 24, 1908, when three months old, showed no abnormal physical signs but weighed only five pounds three ounces. (Birth weight, five pounds.) A week later developed physical signs in lungs. Temperature range very irregular, from 98° to 103°. Died November 10, 1908.

Autopsy. Anatomical diagnosis, acute tuberculosis of pleura, lungs, spleen, liver, pancreas, lymph nodes and intestine. Lungs, pleural adhesions between left upper lobe and chest wall, organized. Entire left upper lobe consolidated. On section it shows cheesy pneumonia. The whole apex is softened, making a fluctuating mass, which has not yet become a cavity. The left lower lobe containing some broncho-pneumonia. The entire right lung is well aerated and closely studded with miliary tubercles. Bronchial lymph nodes, two on the right side measure one-half inch in length and encroach on the oesophagus. On the left side none are larger than one-fourth inch, but all contain cheesy areas. Thymus normal, but surrounded by cheesy mediastinal lymph nodes. Heart normal. Foramen ovale closed. Stomach normal. Kidneys and suprarenals normal. Pancreas, cheesy tubercles on anterior surface, near upper border. Spleen enlarged, contains a number of cheesy tubercles one-sixteenth inch in diameter. Liver deeply congested, contains about 20 tubercles one-sixteenth inch in diameter. Duodenum normal. Jejunum, six round typical tuberculous ulcers, none of which involve the peritoneal coat. Ileum, nine small round ulcers, two within a Peyer's patch. Mucous membrane between lymph follicles pale, but otherwise show no change. Large intestine, mucosa markedly congested. There are no ulcers, but the walls are slightly thickened and the solitary follicles are all enlarged. Mesenteric lymph nodes almost all cheesy and enlarged. None are more than one-fourth inch in diameter.

GENERALIZED TUBERCULOSIS—CHILDREN, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
382	Mesenteric Nodes	H. Type 1	Non-virulent	F.B.	4 1/6 yrs.	F.	2 1/2 mos.	Generalized Tuberculosis

(382) History. Mother coughed for 13 months before her death, lost weight, and expectorated, but there were no hemoptyses. Breast fed for two months; then received condensed milk for a year. Measles five months ago, April, 1908; has lost weight since. The present illness began eight weeks ago with weakness, loss of appetite, vomiting, irregular stools and evening temperature. The abdomen has been swollen for the past six weeks. There is pain in the abdomen occasionally during the day. The child is up and around, but stupid. Temperature range from 98° to 102.6° with marked remissions. Leucocyte count 15,300 October 29, 1908, operation. Coeliotomy. Several small tubercular nodules on the omentum and on the bladder and several small caseous masses in the mesentery. Oxygen allowed to flow in. Died December 17.

Autopsy. Anatomical diagnosis, primary intestinal tuberculosis. Chronic tubercular peritonitis, atelectasis, acute miliary tuberculosis of the liver, spleen and bronchial lymph nodes, chronic ulcerative tuberculous entero-colitis, cheesy tuberculosis of mesenteric lymph nodes. Lungs, no pleurisy; no pneumonia. There is an area of atelectasis in the right upper lobe extending throughout the whole lower half. Bronchial lymph nodes, the largest is three-eighths inch in diameter and contains only young tubercles. None are cheesy. They are somewhat pigmented. Liver moderately congested with a mass of miliary tubercles over the right lobe, where it is adherent to the abdominal wall. There are very few tubercles in the liver substance. Spleen, 4 x 2 inches. Firm and studded with early miliary tubercles. Perisplenitis. Stomach, normal. Suprarenals and pancreas normal. Kidneys, acutely congested. No tubercles. Peritoneum, no fluid. Both parietal and visceral surfaces are studded with yellow tubercles varying from 1 to 6 m.m. in diameter. The adhesions between the intestinal coils are not marked, except over the cecum and sigmoid flexure, where they are too dense and thick to be readily separated. Over the entire small intestine there are only small tubercles and recent fibrin. The cecum and appendix are bound down by a dense inflammatory mass in which large, cheesy lymph nodes are embedded. On separating this mass and opening into the ileum, just above the cecum, a perforated ulcer is found in the lowest Peyer's patch. Another such perforation is found a little higher up. The wall of the cecum is greatly thickened and the mucosa is covered with large ulcers. The rectal wall is less thickened. The upper ileum is of normal thickness. The mass of mesenteric lymph nodes is in two halves: one adherent to the cecum and measuring 6 x 4 cm.; the other adherent to the sigmoid flexure and measuring 5 x 4 cm. Both are hard and composed of single nodes, 0.5 to 2 cm. long. All these nodes are cheesy, but not softened. The appendix is not perforated.

GENERALIZED TUBERCULOSIS—CHILDREN, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
388	Bronchial Nodes	H.Type 1	Non-virulent	J.A.	11½ mos	M.	?	Generalized Tuberculosis
389	Mesenteric Nodes	H.Type 2a	"					
392	Bronchial Nodes	H.Type 1	Non-virulent	R.P.	2¼ yrs.	M.	?	"

(388) History. Bottle fed—cows' milk. Gastro-enteritis, with loss in weight. Cervical nodes palpable. Temperature range 98.8° to 102°. Cough and dyspnoea. Died.

Autopsy. Anatomical diagnosis, tuberculosis of pleura, lungs, lymph nodes, spleen, liver, peritoneum, intestine. Pleura, tubercles scattered over parietal layer. Lungs, left lung, no consolidation; numerous pinhead tubercles scattered through its substance. Right lung shows caseous pneumonia in its upper lobe, with a cavity at the apex containing cheesy material and measuring about 1x½ inches. This region is especially adherent to the chest wall. Both middle and lower lobes show areas of caseous pneumonia. Small tubercles are found over the entire surface of the lung. Bronchial lymph nodes, on the right side, are very large and caseous; on the left side they are swollen and contain small tubercles. Thymus, small. Liver, normal substance, with miliary tubercles scattered over the surface. Spleen measures 2x1½ inches. It is friable and red, with many tubercles on its surface and in its substance. One tubercle measures 2 m.m. Heart normal. Stomach, normal. Duodenum and jejunum, normal. In the lower fourth of the ileum there are four small ulcers, with an area of congestion surrounding them. Large intestine, the solitary follicles are swollen throughout, and in six there is definite ulceration. There is no special thickening of the walls. Mesenteric lymph nodes, all enlarged, many of them showing small areas of caseation. Suprarenals, kidneys and pancreas normal. Retroperitoneal lymph nodes enlarged, two showing definite caseation.

(392) History. Breast fed until the 15th month. Two weeks ago, November 2, 1908, began to have cough and dyspnoea. Loss in weight. Cervical nodes, palpable. Abdomen, distended. Cervical Potts Disease. Died November 17, 1908.

Autopsy. Anatomical diagnosis, caries of spine, miliary tuberculosis of lungs, liver, spleen and lymph nodes. Tuberculous ulceration of intestine. Atelectasis. Heart normal. Foramen ovale closed. Lungs, no pleurisy. Miliary tubercles numerous in both lungs. No cheesy material and no pneumonia. Area of atelectasis in right lower lobe. Bronchial nodes, largest on right side, where one is three-fourths inch in length and cheesy and dry on section. The others contain small tubercles only. Spleen, normal size with many tubercles in its substance. Liver, normal size. Congested with a few tubercles in its substance. Stomach, normal. Jejunum and ileum contain about six round typically tuberculous ulcers, which do not involve the peritoneum. Colon, mucosa congested. No ulcers. Mesenteric nodes all enlarged. A few contain cheesy tubercles. Suprarenals, kidneys and pancreas normal. Vertebral column, there is an abscess containing cheesy material, in front of the bodies of the fourth to eighth dorsal vertebrae. Denuded bone is encountered on incising this. The body of the seventh dorsal vertebra is almost entirely destroyed, while the fifth and sixth are not so extensively involved.

GENERALIZED TUBEROULOSIS—CHILDREN, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
434	Inguinal Nodes	H. Type 1	Non-virulent	A.H.	11 mos.	M.	6 wks.	Generalized Tuberculosis
435	Bronchial Nodes	"	"					

(434) History. An aunt whom the child frequently visited died eight months ago of an active pulmonary tuberculosis. Breast fed. About three weeks ago, October 15, 1908, the mother noticed that the abdomen was swollen and painful on pressure. There was loss in weight. Lymph nodes in groin and in neck palpable. Temperature range within $1\frac{1}{2}^{\circ}$ of normal. The abdomen remained distinctly distended throughout the illness and death occurred on November 23, 1908.

Autopsy. Lungs very firm, adhesions between left lung and chest wall. Left lung, upper lobe almost completely solid, showing on section a broncho-pneumonia with a calcareous mass in the center. Lower lobe and lingula are well aerated. Right lung pale. No pleurisy. No pneumonia. Bronchial lymph nodes all enlarged. Those on the left side are completely cheesy; on the right side they are enlarged but not cheesy. Mediastinal nodes much enlarged and some of them cheesy. Pericardium, adherent to the left pleura. Heart normal. Foramen ovale closed. Abdomen, liver very adherent to diaphragm and to abdominal wall. Glisson's capsule is markedly thickened, and in it are cheesy tubercles varying in size from a pin-head to three-eighths inch. Tubercles are also very marked along the round ligament and on the gall bladder. The abdominal wall is four times its normal thickness, owing to œdema and the formation of innumerable tubercles.

TUBERCULAR MENINGITIS*—CHILDREN

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
2	Sputum	H. Type 1	Non-virulent	M.L.	5 mos.	F.	1½ mos.	Tubercular Meningitis
4	Cerebro-spinal Fluid	"	"					
61	"	"	"	M.S.	10 mos.	M.	5 wks.	"
72	"	"	"	W.B.	15 "	M.	7 days	"

- (2) History. Breast fed. No tuberculosis in the house. The illness began December 15, 1907, with fretfulness and crying. Retraction of the head, clenching of the hands and strabismus began January 1, 1908. The child gradually became stupid and lay quiet, screaming, however, when the head was touched. Physical examination showed equally dilated pupils, which reacted to light, and marked internal strabismus. Lung, heart and abdomen negative. Head retracted, knee jerk much exaggerated. Thirty c.c. of bloody fluid were withdrawn by lumbar puncture February 2, 1908, and the following day 50 c.c. of turbid fluid, which contained tubercle bacilli. The child now began to cough and tubercle bacilli were demonstrated in the sputum. Convulsions appeared, followed by coma, and death on February 8, 1908. Temperature range 98° to 101°. No autopsy.
- (61) History. Breast fed. Early in February the child began to cough and to have a high fever. Vomiting appeared, followed after an interval of a few days by convulsions. Physical examination showed the child to be drowsy but conscious, with marked internal strabismus. Lungs, many rales; no dullness. Heart a little irregular. Abdomen, negative. Marked general rigidity. Kernig's sign present. Lumbar puncture; withdrew 60 c.c. of clear fluid, which contained tubercle bacilli. Death occurred, preceded by coma, on March 18, 1908. Temperature range 100° to 104°. No autopsy.
- (72) History. Has been growing drowsy since April 3d. Rapidly became comatose and hemiplegic. Retraction of neck, and positive Kernig's sign. Lumbar puncture; withdrew 35 c.c. of clear fluid, in which no tubercle bacilli could be found. Died April 11, 1908. No autopsy.

* The cases are classified as simply tubercular meningitis in the absence of data as to how generalized a tuberculosis was present.

NOTE.—See Generalized Tuberculosis, Children, Nos. 58B, 74, 148 for other cerebro-spinal cultures.

TUBERCULAR MENINGITIS—CHILDREN, Cont'd

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
306	Cerebro-spinal Fluid	H. Type 1	Non-virulent	D.K.	7 yrs.	M.	1 mo.	Tubercular Meningitis
443	"	"	"	I.S.	11 mos.	F.	7 days	"

- (306) History. Tubercular family history and history of contact both denied. Breast fed for 16 months; this feeding, however, having been supplemented by milk from a single cow. Measles at four years, no sequelæ. Two or three attacks of swollen lymph nodes, each one lasting for a few days, and generally involving the cervical nodes. Once, however, those in the axilla were concerned. Ten days before admission to the hospital he began to have headache, anorexia and vomiting, with abdominal pain and loss of flesh. The headache was worse at night. There were neither projectile vomiting nor sudden sharp outcries. Upon admission, October 12, 1908, the right pupil was larger than the left. Kernig's sign was marked, and there was tenderness over the cervical and upper dorsal vertebræ, with tension of the posterior muscles of the neck. The lungs were negative. About 10 c.c. of clear fluid was aspirated from the cerebro-spinal canal. After admission the child had all the signs and symptoms of cerebro-spinal meningitis, and died November 3, 1908. No autopsy.
- (443) History. Breast fed. Illness began December 11, 1908, with drowsiness. When awake the child was very restless. Vomiting and constipation were present. At first there were no eye symptoms and no convulsions. Examination, lymph nodes of neck and groin palpable. The skin is almost cyanotic and tache cerebrale is present. The pupils are irregular in reaction and unequal in size. The arms, legs and neck are rigid, but there is no opisthotonos. Respiration is irregular and at times shallow and rapid. At other times it is slow and deep. A few rales are heard in the region of the right nipple. The heart is normal and its action good. The abdomen is negative. Fifteen c.c. of clear fluid, containing tubercle bacilli, were obtained by lumbar puncture. The child grew progressively worse. Convulsions supervened, followed by coma and death, December 18, 1908. Temperature range 98° to 102.4°. No autopsy.

ABDOMINAL TUBERCULOSIS—CHILDREN

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
109*	Mesenteric Node	B. Type 1	Virulent	Baby W.	23 mos.	?	4 mos.	Tubercular Peritonitis
155	Cervical Node	H. Type 1	Non-virulent	M.L.	6½ yrs.	F.	{ 3¼ yrs. 4⅙ yrs. }	Tuberculosis of Cervicals Axillary and Mesenteric Nodes
604	Retrocecal Node	"	"					
433	Appendicular Node	B. Type 1	Virulent	R.D.	8 yrs.	F.	?	Tuberculosis of Mesenteric Nodes (of appendix)

(109) *History. Breast and certified milk for 18 months, upon which the baby thrived. The food was then changed and ordinary milk substituted. Shortly after this the child began to fail and developed symptoms of tubercular peritonitis. No tuberculosis suspected elsewhere. A coeliotomy was performed; miliary aubercles were found, extensively involving the peritoneum, and there was a great mass of omentum in the region of the ileo-cæcal valve. The child did not rally from the operation. Died soon afterwards. No autopsy.

(155) History. Mother tuberculous. In February, 1905, swelling noticed in left side of neck and an abscess formed. These nodes were excised April, 1907. The child was re-admitted to the hospital, February, 1908, and enlarged nodes were found in both sides of the neck, varying in size from size of shot to size of olive. In the right axilla a fluctuating mass present, the size of an orange. This axillary mass was removed. Later, June, 1908, the nodes from the right side of the neck were excised (155), the tonsillar node was the size of a lemon, the remaining nodes varying in size from pea to olive size. All showed tubercles and caseation. While under the anæsthetic, the mesenteric nodes were found palpable. In February, 1909, pain developed in the right groin. On examination, few discrete nodes palpable in cervical region. Abdomen rigid and mass felt in lower right quadrant. A laparotomy was performed. Some free peritoneal fluid present and a large retrocecal abscess and mass of caseous broken down nodes. These were removed (604). No evidence of involvement of lungs. Discharged April 21, 1909, improved, sinus still discharging.

(433) History. Three years ago she suffered an acute attack of appendicitis which lasted two or three days. A similar attack began a week ago, ushered in by pain and vomiting. The attack was not very severe, and there were no urinary symptoms. It lasted for about a week. Examination before operation was entirely negative. Appendectomy. Several nodes in the mesentery of the appendix, each about the size of a bean, were excised. Discharged, cured, one month after operation. Pathological examination, advanced tuberculosis of lymph nodes. Chronic appendicitis. No tuberculosis of the appendix.

* The milk from the dealer who supplied the milk for this child was subsequently tested and tubercle bacilli were isolated.

Note.—See "Generalized Tuberculosis," Nos. (16 and 19) 62, 69 (97, 99 and 100), 122, 274, 276, 291, 377, 382, 389 for other cultures from abdominal organs.

TUBERCULOSIS OF BONES AND JOINTS.

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
202	Pus	H.Type 4	Non-virulent	M.S.	?	?	?	Tuberculosis of Hip
203	"	H.Type 1	"	E.S.	?	?	?	"

(202) History. Not obtainable.

(203) History. Not obtainable.

GENITO-URINARY TUBERCULOSIS.

No.	Material.	Cultures.	Rabbit Virulence.	Name.	Age.	Sex.	Duration Disease.	Diagnosis.
199	Kidney	B.Type 1	Virulent	L.C.	26 yrs.	F.	3 mos.	Tuberculous Kidney
215	Urine	H.Type 1	Non-virulent	?	?	?	?	?
372	"	"	"	Miss S.	21 yrs.	F.	6 mos.	Tuberculous Kidney

(199) History. Began April 4, 1908, suddenly with frequent micturition, pain in the bladder and attacks of pain in the region of the right kidney. No tuberculosis elsewhere. Catheterization of the left ureter resulted in the withdrawal of normal urine, while that from the right ureter contained pus cells, red blood cells, casts and tubercle bacilli. The right kidney was removed July 7, 1908. About one-fifth of the kidney substance had been converted into an abscess which opened into the pelvis of the kidney. Scattered through the rest of the organ, but more especially in the region of the abscess, were small tubercles. Left the hospital and has been apparently perfectly well since the operation.

(215) History. Not obtainable.

(372). History. Has always been strong and well. There is no tuberculosis in her family. About six months ago there appeared an acute cystitis which lasted for 10 days, and during which there was some slight hæmaturia. About two months later the symptoms recurred. This time they were much worse and lasted six weeks. The patient seems to be in good condition. An acid fast bacillus was found in the urine.

SUMMARY OF RESULTS

Diagnosis of Cases Examined*	Adults (16 yrs. or over)		Children (5 to 16 yrs)		Children (under 5 yrs.)	
	Human.	Bovine.	Human.	Bovine.	Human.	Bovine.
Pulmonary Tuberculosis...	225	—	8	—	2	—
Tuberculous Adenitis (Cervical).....	2	—	14	4	4	6
Tuberculous Adenitis (Inguinal).....	—	—	2	—	—	—
Generalized Tuberculosis..	2	—	1	—	16	4
Tubercular Meningitis.....	—	—	1	—	4	—
Abdominal Tuberculosis...	—	—	1	1	—	1
Tuberculosis of Bones and Joints.....	—	—	2	—	—	—
Genitourinary Tuberculosis	1	1	1	—	—	—

For ease of reference to the bovine viruses the following list is appended. They are classed as bovine by reason of their cultural characteristics and virulence for rabbits.

Tuberculous Adenitis (children), 96, 163, 184, 216, 217, 247, 260, 262, 381, 393.

Generalized Tuberculosis (children), 62 (63 and 69), 66 (122 and 123).

Abdominal Tuberculosis (children), 109, 433.

Genito-urinary Tuberculosis, 199.

*In a few instances where the age is not accurately known, the approximate age has been used in this tabulation, though the age is given as (?) in the other tables.

VIRUSES FROM MILK

No.	Material.	Cultures.	Rabbit Virulence.	Remarks.
8	Milk	B. Type 1	Virulent	
12	"	"	"	
14	"	"	"	
89	"	"	"	
111	"	H. Type 1	Non-virulent	Human Type, apparently market contamination

VIRUSES—BOVINE ORIGIN (Controls from cattle)

259	Lung	B. Type 1	Virulent	
271	Lymph Node	"	"	
310	Ing. Node	"	"	
311	Mes. Node	"	"	
312	Bron. Node	"	"	
313	"	"	"	
314	"	B. Type 3	"	
315	"	B. Type 1	"	
317	"	"	"	
321	Lung	"	"	
322	Bron. Node	"	"	
323	"	"	"	
324	Lung	"	"	
327	Bron. Node	B. Type 3	"	
329	Tissue	B. Type 1	"	
330	"	"	"	
331	"	"	"	
332	"	"	"	
334	"	B. Type 2	"	
489	Bron. Node	B. Type 1	"	
490	"	"	"	
491	"	"	"	
493	"	"	"	
495	Lung	"	"	

SUMMARY OF CALF INOCULATIONS

Virus.	Diagnosis.	Weight of Calf	Autopsied after.	Weight at Autopsy.	Autopsy.
No. 64 (See No. 125) Tonsils	Pulmonary Tuberculosis (Adult)	190 lbs.	142 days (killed)	267½ lbs.	Gain in weight, 77½ lbs. Caseous area at site of injection. No other tuberculous lesions.
No. 118 Sputum	"	185½ lbs.	140 days (killed)	269 lbs.	Gain in weight, 83½ lbs. Caseous area at site of injection. Tubercles in preapular lymph node.
No. 15 Sputum	Pulmonary Tuberculosis (Children)	119 lbs.	142 days (killed)	174 lbs.	Gain in weight, 55 lbs. Caseous area at site of injection. No other tuberculous lesions.
No. 116 Cerv. Nodes	Tuberculous Adenitis (Children)	189½ lbs.	142 days (killed)	273 lbs.	Gain in weight, 83½ lbs. Caseous area at site of injection. No other tuberculous lesions.
No. 150 Cerv. Nodes	"	218 lbs.	142 days (killed)	284 lbs.	Gain in weight, 66 lbs. Caseous area at site of injection. No other tuberculous lesions.
No. 155 Cerv. Nodes	"	263 lbs.	142 days (killed)	372 lbs.	Gain in weight, 109 lbs. Caseous area at site of injection. No other tuberculous lesions.
No. 163 Cerv. Nodes	"	156 lbs.	73 days (died)	126 lbs.	Loss in weight, 30 lbs. Progressive generalized tuberculosis.
No. 217 Cerv. Nodes	"	203½ lbs.	23 days (died)	134 lbs.	Loss in weight, 69½ lbs. Progressive generalized tuberculosis.
No. 262 Cerv. Nodes	"	158½ lbs.	53 days (died)	136 lbs.	Loss in weight, 22½ lbs. Progressive generalized tuberculosis.
No. 19 Spleen	Generalized Tuberculosis (Children)	262½ lbs.	140 days (killed)	358 lbs.	Gain in weight, 95½ lbs. Caseous area at site of injection. No other tuberculous lesions.
No. 20 (See 16) Bron. Nodes		133 lbs.	140 days (killed)	195 lbs.	Gain in weight, 62 lbs. Pocket of fibrous tissue at site of injection, surrounded by caseous cervical nodes.

SUMMARY OF CALF INOCULATIONS

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Virus.	Diagnosis.	Weight of Calf.	Autopsied after.	Weight at Autopsy	Autopsy.
No. 58B (See 58A) Cer. Sp. Fl.	Generalized Tuberculosis (Children)	129½ lbs.	140 days (killed)	204 lbs.	Gain in weight, 74½ lbs. Caseous area at site of injection. No other tuberculous lesions
No. 62 Mes. Nodes	"	125 lbs.	125 days (killed)	206 lbs.	Gain in weight, 81 lbs. Regressive generalized tuberculosis. Generalization marked.
No. 74 Cer. Sp. Fl.	"	157 lbs.	140 days (killed)	204 lbs.	Gain in weight, 47 lbs. Caseous area at site of injection. Mass of caseous nodes in ligament at junction of two gastric pouches.
No. 98 (See No. 97) Lung	"	204 lbs.	142 days (killed)	288 lbs.	Gain in weight, 84 lbs. Two caseous areas at site of injection. No other tuberculous lesions.
No. 99 Spleen		242½ lbs.	142 days (killed)	313 lbs.	Gain in weight, 70½ lbs. Caseous area at site of injection. No other tuberculous lesions.
No. 100 Mes. Nodes		226 lbs.	142 days (killed)	332 lbs.	Gain in weight, 116 lbs. Caseous area at site of injection. No other tuberculous lesions.
No. 148 Cer. Sp. Fl.	"	190 lbs.	142 days (killed)	246 lbs.	Gain in weight, 56 lbs. Caseous area at site of injection. No other tuberculous lesions.
No. 2 (See No. 4) Sputum	Tubercular Meningitis	197½ lbs.	142 days (killed)	267 lbs.	Gain in weight, 69½ lbs. Caseous area at site of injection. No other tuberculous lesions.
(No. 61 Cer. Sp. Fl.	"	109 lbs.	140 days (killed)	142 lbs.	Gain in weight, 133 lbs. Caseous area at site of injection. No other tuberculous lesions.
No. 109 Mes. Nodes	Tubercular Peritonitis (Child)	152 lbs.	24 days (died)	120½ lbs.	Loss in weight, 31½ lbs. Progressive generalized tuberculosis.
No. 310	Bovine Controls	218 lbs.	20 days (died)	163 lbs.	Loss in weight, 55 lbs. Progressive generalized tuberculosis.
No. 491	"	121 lbs.	40 days (died)	110 lbs.	Loss in weight, 11 lbs. Progressive generalized tuberculosis.

THE INCIDENCE OF TUBERCLE BACILLI IN NEW YORK CITY MILK

With a Study of Its Effects on a Series of Children

ALFRED F. HESS, M.D.

One of the most important questions to-day which confront those fighting the spread of tuberculosis is: What proportion of infection is human, what proportion bovine in origin? It is not necessary for me to review in this place the various answers that are given this question by sincere and capable workers in the same or different countries. In the United States we find Theobald Smith¹ stating that, in his opinion, not over 1 per cent. of human tuberculosis is of bovine origin, and, on the contrary, others—for example, Ravenel²—believing that a considerable percentage of the disease is of this origin. The British Tuberculosis Commission recently reported that “a very considerable amount of disease and loss of life, especially among the young, must be attributed to the consumption of cow’s milk containing tubercle bacilli.” The opposite view of Professor Koch is known to all. In view of such conflicting statements on a subject of such vital importance, physicians and laymen are, at the present time, undecided whether they should concentrate their energies against the dangers from the tuberculous individual, or whether they must guard against a dual source of infection. Prophylaxis suffers from this indecision.

Scope of the Investigation

My work was undertaken with a threefold object: 1, probing one of the elemental phases of this broad question, to ascertain how often milk supplied to a large city, such as New York, contains virulent tubercle bacilli; 2, to study the nature of these bacilli, to learn whether they conformed uniformly to the bovine type as was to be expected considering their source; and, finally, 3, to report on the health of some children who consumed milk containing virulent bacilli.

1. Smith, Theobald: The Channels of Infection of Tuberculosis, Boston Med. and Surg. Jour., Sept. 26, 1907.

2. Ravenel: Etiology of Tuberculosis, Am. Jour. Med. Sc., October, 1907.

I realized that the presence of tubercle bacilli in milk did not necessarily furnish an index of the frequency of infection of those who drink such milk. Nevertheless, it shows the frequency of exposure to this infection, so that it is important that we should at the outset have an approximate idea of the incidence of such bacilli in the market milk. I found experiments of this nature few in number and conflicting in results. The milk of New York City had never been investigated in this particular, and examinations which had been made elsewhere were, in the main, incomplete or dated back to a period when our milk was obtained under quite different conditions from those which exist at the present time. However, since I began this investigation more than a year ago, a comprehensive report of the tuberculous contamination of the milk supply of the city of Washington has appeared,³ to which I shall refer in detail. The literature of this subject will be found well abstracted in this publication.

As I have mentioned, the second part of my investigation consisted in isolating in pure culture some of the tubercle bacilli found in the milk, and in differentiating them according to type. This was undertaken in order to learn whether all the cultures would prove to be bovine, or whether possibly we might find human or intermediate varieties. It seemed also desirable that more strains should be isolated from bovine material, in order to judge more certainly of the distinction between the two types of bacilli.

The material was obtained in the following manner: In the tests of raw milk, specimens were gathered only from the large 40-quart cans and not from bottled milk. This was done, partly because the facilities of the health department rendered it simpler, but more especially because more than one-half of the milk sold in New York is not bottled, but constitutes the so-called "loose milk." The fact that unbottled milk forms such a large proportion of the municipal supply is sufficient in itself to warrant its selection as a basis for examination. Furthermore, loose milk and bottled milk are frequently identical, are dispensed by the same dealer and derived from the same dairy. The specimens were collected in the same manner as those brought to the

3. Anderson: U. S. Hyg. Lab. Bull. 41.

laboratory twice a week for general bacteriologic examination. I used the same sterilized 1-ounce bottles with a tight-fitting tin cap. The milk was taken indiscriminately from grocery stores, dairies and large plants in various quarters of the city. Two specimens were dipped out from each can, one for the bacterial count, another for examination for tubercle bacilli. Six to twelve were collected at a time for my purposes, although one hundred were obtained by the inspectors for general examination. In most cases I obtained the milk myself, and my selection was guided merely by an attempt to obtain specimens from dealers who had small children who drank this milk in a raw state. This was done with the object of determining the subsequent health of these children, which study forms the basis of the third part of this paper.

Investigation of Tubercle Bacilli in Milk

In the laboratory the specimens were kept in the refrigerator. Before being opened they were thoroughly shaken and then 10 c.c. of milk were removed by means of a sterile pipette and transferred to a small tube, in which it was centrifuged from five to seven minutes. Two smears were made from the surface of the cream which formed a solid layer at the top of the tube. The entire cream layer, which varied in amount, was removed by means of a sterile copper spoon and placed in a Petri dish. The skimmed milk was poured away and disregarded with the exception of the lowest cubic centimeter, which was poured into another dish. Two smears were made from the dregs in the centrifuge tube.

These two portions of milk, the cream and the lowest cubic centimeter, were drawn into small antitoxin syringes and injected into two guinea-pigs. It was necessary to dilute the cream with a small quantity of salt solution in order to render it fluid. As far as possible unused pigs of medium weight were employed; in many instances, however, I had to use larger pigs which had served as tests for diphtheria antitoxin. In order to make certain that such pigs were not previously tuberculous, autopsies were performed on fifty which had been used in this work. In no instance was any tuberculous infection encountered. In the early tests the inoculations were made into the

muscles of the thigh, following the recommendation of Ostertag.⁴ Later, however, subcutaneous inoculations into the groin were resorted to, as the disadvantages of the intramuscular method outweighed its advantages. It was found that the deep injections at times gave rise to hematomata at the site of inoculation, that they were sometimes followed by sudden death, and that the inguinal glands enlarge less markedly than when the pigs were inoculated subcutaneously. For each inoculation a different syringe and needle were used. The pigs inoculated on the same day were kept in the same cage. Very few abscesses developed, probably owing to the fact that almost three-quarters of the inoculations were made deep into the muscles; those which did form did not ulcerate through the skin. The animals were examined twice a week. They were not killed after a definite period, but in some instances, in which cultures were not desired, they were allowed to live long after they were known to be tuberculous. In this way we could judge somewhat of the dosage and the virulence of the tubercle bacilli in the milk.

One hundred and twelve specimens of raw milk were tested in the manner described, but as in five instances the animals died within two weeks of inoculation or were lost in other ways, only 107 came under consideration. Table I gives a summary of the results. Nineteen specimens were found to contain tubercle bacilli. It was found, however, on tracing all these samples to their source, that two were duplicates, so that we must consider that the positive inoculations numbered only seventeen. Thus the figures resolve into seventeen positive tests out of 107, which means that 16 per cent. of the milk contained tubercle bacilli.

4. Ostertag: Untersuchungen über die klinische und bakteriologische Feststellung der Tuberkulose des Rindes, 1905.

TABLE 1.—DATA OF TUBERCULOSIS MILK SPECIMENS.

Date.	Tuberculous Specimens.	Tuberculous Sediment or Cream.	Bacteria per c.c.	Designation of Wholesaler.	Wholesalers Who Sell Similar Milk in Bottles.
10/24	I	*Sediment	390,000	A	O
11/7	I	"	4,800,000	B	I
	I	Cream and Sed.	80,000	C	I
11/12	I	Cream	170,000	W	I
	I	Cream and Sed.	90,000	W	I
11/19	I	" " "	80,000	E	I
	I	" " "	00,000,000	B	I
11/25					
12/5	I	Sediment	50,000	O	O
	I	Cream	1,400,000	G	I
	I	" and Sed.	20,000	B	I
	I	"	60,000	B	I
	I	*Sediment	00,000,000	B	I
12/19					
12/26					
12/31	I	*Sediment	00,000,000	E	I
	I	Cream and Sed.	8,000,000	H	O
	I	*Cream	230,000	B	I
1/6	I	" and Sed.	160,000	J	I
	I	" " "	180,000	J	I
	I	*Cream	60,000	J	I
	I	" and Sed.	00,000,000	J	I
	19				
Excluding dupli- cates	17				

In every one of these seventeen cases, as well as in the two duplicates, tubercle bacilli were found in the guinea-pig at autopsy. The case was looked on as negative unless the bacilli were shown to be present. For example, the autopsy of pig 436, killed three months after inoculation, reads as follows:

At the time of autopsy these lesions were considered definitely to indicate tuberculosis, but as bacilli could not be found, and reinoculation tests proved inconclusive, the case was rejected. In most instances reinoculation into a second series of pigs was made (Table 2). In order to control the results still further and avoid the possibility of confusing the tubercle bacillus with similar acid-fast organisms, cultures were made in each instance. In those cases in which serum or

*Animal inoculated with other part of milk lost or died.

egg media was not employed—in other words, in which the object was not to cultivate the tubercle bacillus—glycerin agar was used. In no instance was an acid-fast organism encountered.

TABLE 2.—DATA OF MILK INOCULATIONS

Date.	No. Samples.	No. Pigs Inoculated.	No. Dying within 14 Days.	Otherwise Lost.	Secondary Inoculations.
10/24	12	24	2	2	2
11/7	12	24			6
11/12	12	24			2
11/19	12	24			4
11/25	6	12			4
12/5	12	24	1		6
12/19	6	12	1		
12/26	6	12	1	1	
12/31	12	24	1	3	
1/6	10	20	4	3	2
2/5	12	24		1	
Total	112	224	10	10	26
Net total	107				

(In five instances both pigs were lost or died.)

Somewhat emaciated, large bilateral inguinal glands; mesenteric glands hard, size of small marble; slight enlargement of iliac and retroperitoneal glands; spleen three times normal, containing a caseating abscess and miliary tubercles. Liver showed miliary tubercles, lung negative.

The smears made from the cream and from the sediment, of which mention has been made before, showed no tubercle bacilli. In no slide could they be definitely made out, although in those instances in which animal inoculations demonstrated tuberculosis the slides were carefully examined for a second time. They showed a large number of bacteria and leucocytes, roughly about an equal number in cream and sediment, but the number was found to bear no relation to the presence or absence of tubercle bacilli in the milk (Table 1).

I next examined some "commercially pasteurized" bottled milk, making use of the same technic. Eight samples were obtained from three different plants, with the intention of using more samples should these first tests give a negative result. The dealers in this milk state that it has been subjected to a temperature of 160° F. for forty seconds. In one of the eight samples tubercle bacilli were demonstrated by ani-

mal inoculation, which proves that this method, in practice, furnishes inadequate protection. Investigation on this particular was pursued no further.

Human and Bovine Tubercle Bacilli in Milk

Before discussing the results which we have outlined above, it may be well to give a brief account of the cultures of tubercle bacilli which were isolated. The details of this laboratory study will be published elsewhere. The method used was that employed by me in former work in this field,^{5 6} For the first generation dog serum or egg medium was used. The morphology of the bacilli, facility for cultivation, and virulence toward rabbits were considered in the differentiation of the human from the bovine type. The ability of a culture to induce a fatal generalized tuberculosis in the rabbit was looked on as evidence of its being bovine in nature. Eight cultures were isolated in all; in two other cases cultures were attempted but failed. From a practical standpoint it is of importance that, although seven proved bovine in type, in one case a human strain was isolated. As far as I am aware this is the first instance in which contamination of milk with the human type of tubercle bacillus has been demonstrated. The actuality of this source of infection, therefore, can no longer be denied; it must be considered in the safeguarding of our milk supply. On the farm, at the dairy, in the retail store, in the varied path from cow to consumer, the tuberculous individual must not have access to the milk.

5. Hess: An Examination of Excised Tonsils. Arch. Pediat., January,

6. Hess: Primary Tuberculosis of the Mesenteric Glands, Am. Jour. Med. 1908, p. 31.

TABLE 3

DATA OF INTRAVENOUS INOCULATION OF RABBITS WITH PURE CULTURES OF TUBERCLE BACILLI

	Total Age of Culture.	Generation.	Age of Culture.	Amount Inoculated.	No. Rabbit.	Result	General Remarks.
Culture I.....	82 days	3d	22 days	0.5 c.c.	320	Chloroformed 21 days (ill)	General Tuberculosis
" II.....	95 "	4th	20 "	0.5 "	321	Died 18 days	"
" III....	76 "	3d	25 "	0.5 "	325	" 16 "	"
" IV.....	67 "	3d	28 "	0.4 "	326	" 20 "	"
" V.....	98 "	4th	21 "	0.5 "	327	" 23 "	"
" VI.....	79 "	3d	30 "	0.5 "	331	Chloroformed 17 days (ill)	"
" VII....	69 "	3d	26 "	0.5 "	332	Died 15 days	"
" VIII }	21 "	3d	63 "	1 mg.	181	Killed 80 days	Few tubercles in lungs and kidneys
	21 "	2d	42 "	1 "	521	Alive 63 days	Gained in weight

Characteristics of the Eight Cultures Isolated

The cultures I-VII must be classed as bovine in type, not only on account of the marked virulence to rabbits which they exhibited, but also because of their cultural characteristics. They grew very sparsely, showing for some generations only a fine veil-like growth, which it was very difficult or impossible to transfer successfully to glycerin broth. They were markedly similar in all these properties, only one standing apart from the others in that it showed a more abundant growth in the first generation. However, even this strain did not grow profusely when compared with cultures of the human variety.

Culture VIII is of especial interest and seems worthy of detailed description. The original material was obtained from a can of milk in a small grocery store. It was inoculated subcutaneously into two guinea-pigs, both of which developed generalized tuberculosis. From one of these animals tissue was inoculated into another pig (No. 855), from which cultures were made upon egg media about two months later. In order not to lose the material, tissue was once more transferred into a pig (No. 972), and further cultures carried out after the same interval. These two sets of cultures proved to be identical; after

three weeks a vigorous confluent growth was obtained on glycerin egg. These results were so unexpected that a rabbit (No. 625) was inoculated with one of these strains and cultures were made from its tissues. By using the rabbit instead of the highly susceptible guinea-pig we made certain of selecting for culture the most virulent bacilli. These strains also grew vigorously in a manner characteristic of bacilli of the human type. Reference to the virulence tests of the table shows that this strain (Culture VIII), in strong contrast to all the others, possessed but feeble virulence for rabbits. One of the rabbits when inoculated weighed 1.740 gm., and when killed had gained 550 gm.; the other weighed 1.710 gm., and now after a period of 63 days, weighs 1.820 gm. Thus this culture, in contradistinction to the others, must be regarded as belonging to the human type, both on account of its facility for cultivation and its comparatively feeble virulence.

From a study of these eight cultures we must emphasize the sharp contrast which usually exists between tubercle bacilli of the human and those of the bovine type, isolated from cattle, a distinction which in the instances here reported was absolutely diagnostic and incapable of misinterpretation. Furthermore the results are instructive from another point of view, for, for the first time, they bring forth an instance where tubercle bacilli of the human variety have been isolated from milk and thus point out another source of danger from contamination by the tuberculous individual.

The percentage of milk found to contain tubercle bacilli, namely, 16 per cent., is so high that we feel that it requires further comment. It must be admitted that the milk chosen for examination, commonly known as "loose milk," is somewhat below average grade. In this fact, however, we can find little comfort, for subsequent inquiry revealed that of the nineteen tuberculous specimens fifteen were sold by dealers who furnish bottled milk coming from the same farms (Table 1). This shows that an examination of the average bottled milk would reveal very nearly the same figures. Another matter of practical interest concerns the diffusion of the bacilli throughout the milk. If several samples were taken from the same can, would all or only some show the presence of tubercle bacilli? This question, which was constantly

in mind, was answered by a chance control experiment. In some instances the samples were taken at random from those sent to the laboratory by the inspectors. It so happened that of the 10 samples submitted on January 6, four proved to be contaminated with tubercle bacilli (Table 2). Recently, on inquiring into the source of these samples, it was learned that the four were taken from two cans in the same plant—in other words, that two were duplicates. This method of control specimens is resorted to by the Board of Health to compare the results of the bacterial counts. In this instance it served as an unconscious control of my work. Of the eight guinea-pigs inoculated with these four specimens, seven developed tuberculosis, one dying prematurely of a secondary infection. As only 10 c.c. of milk were used for the test in each instance, that is, less than one tablespoonful, this would lead us to conclude that these two 40-quart cans were thoroughly permeated with tubercle bacilli, and that there was little possibility of any one partaking of the milk without at the same time partaking of virulent bacilli. Some other of our results lead to the same conclusion: The fact that on November 12 (Table 1) two positive specimens were obtained which emanated from the same wholesaler, although retailed by different stores on the same block, and that on December 5 this was the case in three instances, demonstrates that a considerable portion of the milk sold by the wholesalers on those dates contained tubercle bacilli.

Without attempting a review of the literature we should like to call attention in this connection to the results of some others in similar investigations. In the comprehensive test of the milk supply of the city of Washington, reported this year by Anderson, 6.72 per cent. of the samples were found to contain tubercle bacilli, a percentage considerably lower than that which I report for New York. This wide variation can be accounted for in part by differences in the technic which was employed. In the Washington tests usually only one animal was used for each sample, and, second, the cream was not injected. Had these methods been followed, our percentage would have been considerably lower. This may be judged by the fact that of the 19 tuberculous specimens only nine produced tuberculosis in both of the in-

jected animals (Table 1). In five instances this was due to the premature death of one of the pigs. It may be said in general that the more animals used for testing each sample the greater the chance of finding tubercle bacilli. The other point of difference in technic is more important. As bacteria collect in the cream as well as in the sediment, it is evident that unless we test both portions some may be overlooked. It will be noticed that in two of our cases bacilli were demonstrated in the sediment alone, in three others in the cream alone (Table 1). The animals injected with the negative portion of these samples developed either no lesions or such as were inconclusive. Had we not injected the cream, three of the positive cases would have been reported as negative, which in itself would have reduced our figures from 16 per cent. to 13 per cent. Moreover, higher percentages than we have obtained have been published by others. For instance, Rabinowitch and Kempner⁷ found, in an examination of 25 samples of Berlin milk, that seven samples, or 28 per cent., contained tubercle bacilli. And again, in an examination by Macfadyen⁸ at the Jenner Institute, 17 among 77 specimens, or 22 per cent., were found to be infected with virulent tubercle bacilli.

Condition of Children Who Drank Tuberculous Milk

So much concerning the laboratory part of this study. We have shown how frequently tubercle bacilli are present in the milk of a great city; how, although generally bovine in type, they may be of the human variety. Let us now consider the condition of some children who drink this milk. As I mentioned above, samples were gathered in most instances from such dealers as had children. Each store-keeper was asked whether there were children in the family, and, if so, whether they were given raw milk from the can in the store. Of the 17 dealers from whom milk was obtained which contained tubercle bacilli, ten belonged to this category. These ten had 18 children. As Table 3 shows, nine of these were two years of age or under, and only one over five years of age. The health of these children has been followed for the past year, that is, since they are known to have ingested virulent tuber-

7. Rabinowitch and Kempner: *Ztsch. f. Hyg.*, 1899, xxxi.

8. Macfadyen: *Lancet*, London, 1899, ii, 849.

cle bacilli. They have been visited by me from time to time, and finally during the past fortnight 16 were tested with tuberculin and subjected to a more or less complete physical examination. The conjunctival tuberculin test was employed, as it was feared that the cutaneous test might not be permitted. The parents were told the reason for this medical supervision, and in only a few cases offered objections. The results, which are given in tabular form (Table 3), were briefly as follows:

TABLE 4—DATA CONCERNING CHILDREN WHO DRANK MILK CONTAINING TUBERCLE BACILLI

Milk Dealer.	No. of Children.	Age Years.	Conjunctival Test.	Condition One Year Later.
A	2	1	—	Healthy
		3	—	"
B	1	1½	—	"
C	2	2	—	Poorly nourished
		4	—	Healthy
W	3	5/12	o	"
		2½	.	
		5	+	Poorly nourished. Physical exam. and family history doubtful.
E	1	½	—	
B	1	2	+	Poorly nourished. Cervical adenitis incised 4 mos. ago. Von Pirquet positive. Family history negative.
G	3	3	—	Healthy
		5	—	"
		9	+	" Family history negative.
B	1	5/12	o	Healthy
B	2	1½	—	Poorly nourished
		3½	+	Healthy. Physical exam. and family history negative.
O	2	2	—	Healthy
		4	—	"
	18		4	

Of the 16 children tested with tuberculin, four reacted; two of these were poorly nourished, but showed no definite signs of tuberculosis; the other two were healthy. One of the cases was of especial interest:

+ = positive reaction.
 — = no reaction.
 o = no test.

This child consumed daily a pint of the poorest milk examined (B. Table 1). She was two years of age, poorly nourished, and five months ago developed a cervical adenitis, which was incised at a dispensary. She reacted to both the conjunctival and von Pirquet tests. No other member of the family appeared to be tuberculous.

We realize that we can not draw sweeping deductions from an experiment of this nature. It lacks the precision of laboratory work, a weakness inherent to a test on the human subject. It may well be said that we know neither how often the children drank milk containing tubercle bacilli nor the number of bacilli consumed. In answer to this we can merely state that 10 c.c. of this milk showed the presence of virulent bacilli; that these bacilli were found to be thoroughly distributed throughout the milk, and that the children in most instances consumed a pint or even a quart of such milk daily. Moreover, as this is the only investigation of the kind which has been made, and which it seems possible to pursue, it seems worthy of consideration.

First and foremost it is evident that as almost all the children who drank the milk were later found to be in good health, we may conclude that milk containing tubercle bacilli does not necessarily, nor even usually, excite tuberculosis. The striking frequency with which we found bacilli would of itself force this conclusion on us. For with 16 per cent. of the "loose milk" infected, if infection were coincident with exposure, it would mean that on an average every child who drank this raw milk daily for a week would show some tuberculous lesion. Tuberculin tests and postmortem examinations on infants prove the fallacy of such reasoning. On the other hand, the occurrence of a case of cervical adenitis, one of the most frequent forms of bovine tuberculosis, in an infant known to have drunk milk containing bovine tubercle bacilli, who reacted to tuberculin although surrounded by no source of tuberculous infection, is certainly suggestive of bovine infection. We hope to follow the course of this child and some of the others so as to judge of their future welfare.

It seems to us, judging from our own investigations and those of others, that, at the present time, the relation of bovine tuberculosis to man may fairly be stated as follows: 1. This type of bacillus, although

less virulent than the human variety, is capable of infecting human beings. 2. Children are more susceptible than adults to bovine infection. Numerous instances of tuberculosis of the tonsil, of the cervical and of the mesenteric glands have been proved to have been incited by the bovine bacillus. Among others, Theobald Smith, Fibiger and Jensen, the German and the British Commissions, and myself,⁵ have reported cases of this nature. Recently I was able to collect reports of 44 cases of undoubted bovine infection of the mesenteric glands, 41 of which occurred in children.⁶ In this connection a case taken from this year's Medical Report of the County of Aberdeen, and sent to me by Dr. Adams, seems worthy of summary. A farm servant who had three daughters, aged 9, 6 and 4 years, lost the eldest on January 7, 1907, with symptoms of meningitis. In the beginning of March the youngest became ill and died on the 18th with similar symptoms. An autopsy showed tuberculous meningitis and tuberculosis of the mesenteric nodes. In the cerebrospinal fluid tubercle bacilli were found. There was no history of tuberculosis on the father's or mother's side. The milk which these children drank came from one cow, which was found to have tuberculosis of the udder, and was killed and shown to be suffering from extensive generalized tuberculosis. Careful cultural examination of bacilli from the cerebrospinal fluid of the child, as well as from the milk and from the mesenteric glands of the cow, were made by Dr. J. Milner Adams, of the University Pathological Department, and all found to be bovine in type and similar in their microscopic, cultural and pathogenic characteristics. Neither this well-studied case nor the figures given above are, indeed, sufficient to prove that bovine tuberculosis is the "great white plague" which we are all fighting, but they do show, without doubt, that this bacillus is fraught with danger to children. Even the staunchest supporter of Koch would not care to have his children drink milk from a cow which he knew to be giving forth tubercle bacilli.

As yet there is no sufficient basis for a statistical estimate of the importance of bovine tuberculosis, but we must remember that even if, with Theobald Smith, we charge it with but 1 per cent. of the total tuberculous infections, this small percentage is sufficient to establish it

as a disease worthy of active recognition. To appreciate the force of the plea for recognition of such a small percentage we have but to reflect that tuberculosis claims in the United States every year approximately 200,000 persons and that the total number of infections amount to twice this number. Thus, this small percentage is sufficient to cause the yearly number of cases of bovine infection of human beings in this country to extend into the thousands. It is solely because this type of tuberculosis is submerged by the 90-odd per cent. of human tuberculosis that it is now threatened with losing its identity. If it were possible to diagnose bovine infections by clinical methods, and if the total number were grouped under a different name, instead of being included in the generic term "tuberculosis," we would have a disease of sufficient magnitude to preclude all questions as to the advisability of active preventative measures. Were there as great a number of human beings infected, for instance, by the foot-and-mouth disease of cattle, the matter would be considered as requiring state or federal attention. So that, to summarize, without ever losing sight of the fact that the bacillus of the human type is the cause of the great scourge, the scourge of tuberculosis, it would seem wise, also, to regard the bovine bacillus as the cause of an unquestionable, although comparatively small number of tuberculous infections.

Finally, a word as to the practical lesson which this investigation teaches. It is not new; it has been emphasized with ever-increasing earnestness by many students of the problem. It is to be hoped, however, that the alarming degree of tuberculous contamination found in the New York City milk, and the concrete figures which I have furnished, may prove an effective argument for those who are striving with the legislators in the cause of pure milk. It may be thought that, as I used mixed milk in the tests, it is possible that the bacilli were, in many instances, given off by one infected cow in a herd and were diffused throughout the supply. We can not well fall back on this comforting explanation, when we reflect that a recent report by Moore⁹ showed that of 421 herds in New York State tested with tuberculin, 302 contained reacting animals. This high percentage of disease har-

⁹ Moore: Can Tuberculosis be Eliminated from Cattle? New York State Jour. Med., 1908, viii, No. 5.

monizes with the high percentage of contamination which I found in the city milk. Again, the investigations of Schroeder¹⁰ showing how frequently feces are present in milk, and how frequently tubercle bacilli are present in the feces of apparently healthy cattle, point clearly to a widespread dairy infection. In view of these facts, it would seem wise as an immediate safeguard, thoroughly to pasteurize or to boil all milk not coming from tuberculin-tested cows. "Commercial pasteurization" does not protect against tubercle bacilli; it should not bear the name of pasteurization, or, at most, this milk might be termed "partially pasteurized milk."

At the same time that we are carrying out these temporary expedients, those who are urging broader remedial measures, such as adequate inspection and testing of the dairy herds, should be accorded the support of physicians and laymen alike. For if New York City had been granted, instead of 47 inspectors of farms, 150 such inspectors, it is certain that 16 per cent. of the milk sold in its precincts could not be found to harbor virulent tubercle bacilli.

Summary

Virulent tubercle bacilli were found in 17 among 107 specimens, that is, in 16 per cent. of the milk retailed from cans in New York City. These bacilli were not remonstrable by direct microscopic examination, but were proved to be present by means of animal inoculation. Cream as well as sediment was found to harbor these organisms, so that in all experiments of this nature these two parts of the milk should be used in making inoculations.

Tubercle bacilli were likewise found in a specimen of "commercially pasteurized" milk, showing that this method, as now carried out, does not insure protection in this particular. It is suggested that, as this name is misleading, only such milk be labeled "pasteurized" as has been heated for a length of time and to a degree of temperature sufficient to render it an absolutely safe food.

When the tubercle bacilli were isolated they were found to be in all but one instance bovine in type. In this instance, however, a human

10. Schroeder: The Unsuspected but Dangerously Tuberculous Cow, Bureau of Animal Industry, Cir. 118.

variety was differentiated, which shows that milk may become infected from tuberculous individuals and that this source of contamination should be guarded against.

A number of infants and young children who drank milk containing tubercle bacilli, when examined one year later, seemed to be in average health. A fourth of the number, however, reacted to tuberculin. One of these subjects was in poor physical condition and had suffered from a recent glandular infection.

Although probably over 90 per cent. of tuberculosis is due to infection from human beings, it is believed that we are not, therefore, justified in neglecting the danger from bovine infection, for even a small percentage of the infections in the United States means thousands of cases of tuberculosis.

As an immediate safeguard, milk not coming from tuberculin-tested cows should be pasteurized or brought to a boil. Many additional inspectors should be allowed for examining the herds, and, finally, all cows should be tested with tuberculin, and animals which react should be condemned or isolated.

ON THE TYPE OF CULTURES FROM OLD CUTANEOUS TUBERCLES OF BUTCHERS

By ALFRED F. HESS, M.D.

The studies thus far carried out in different countries, using the same methods of differentiation, agree in charging the human type of tubercle bacillus with an overwhelming preponderance of human infections. In this connection, however, there is one disturbing criticism that is now and again brought forth to shake the confidence imposed in these conclusions, based as they are on the differentiation of the types of bacilli. It is claimed by some capable workers that it is possible for the bovine bacillus to be so altered by years of sojourn in the human tissues that its essential characteristics become altered and it simulates the human type of bacillus. Without doubt if this metamorphosis can readily take place some of those infections which we now believe to have been of human origin may well have had their source in bovine infections of years ago, and it is to no purpose to designate them as human. In fact, if this be so, the entire method of differentiation which has accomplished so much in the past decade and promises so much more in the practical and theoretical study of tuberculosis, would have to be abandoned as not only unreliable but misleading.

The main difficulty in studying this question in man is that we are rarely certain of the nature of the original infection and accordingly cannot judge of the change of type. Our main reliance is generally circumstantial evidence. An exception to this rule, however, is furnished by those few cases of inoculation tuberculosis where the bovine source of infection is definitely known. I have with difficulty found two cases of this nature, both in slaughter-house workers, and report a study of them coupled with some other experiments relating to the stability of the types of tubercle bacilli.

Case 1—This man had been employed in his present trade for seven years. Four years ago, while slaughtering an animal, he cut the middle finger of his right hand on the pointed end of a rib. He gave no thought to this injury until he found that it was slow to heal; he then merely applied various ointments. Gradually a nodule appeared at the

site of the wound which assumed its present condition. He was aware of the tuberculous nature of this nodule, and in fact directed me to another worker who had the same skin lesion. I shall not enter into a minute description of the appearance of this nodule. It was the size of a large pea, brownish red and very firm in consistency. The glands at the elbow and axilla were not enlarged. The general health of the man was excellent.

Case 2—This case was very similar to the previous one. This man while slaughtering a tuberculous cow cut himself with a knife across the knuckles. This accident happened about six years ago. He remembered the date, as he was forced by this disability to quit work for some weeks and to enter the employment of another firm. As in the previous instance, a nodule appeared at the site of injury. The nodule was situated over the fourth left metacarpal joint, and resembled the tubercle described in the previous case, excepting that its surface was rougher and papilliform. This man also was healthy to all appearance; however, a large hard epitrochlear gland was palpable at the right elbow.

Both of these men were induced to have these nodules excised. After excision the surface of the growths was removed, and they were thoroughly washed in sterile water. Two guinea pigs were inoculated subcutaneously with each growth. All four animals developed tuberculosis. The cultural growth from these two cases proved to be very similar, in fact I could not distinguish between them. They both grew sparsely on egg media. They also showed a high degree of virulence for rabbits producing a generalized tuberculosis when inoculated intravenously in the dose of 1 mg. In other words, they were typically bovine.

Before discussing these results I shall describe an experiment undertaken with the object of transforming the bovine type of the tubercle culture medium resembling in its composition as closely as possible the human tissues. From the outset the inherent variance between an artificial culture medium and the living tissues was clearly realized. For this purpose, however, what may be termed a human placenta glycerin broth was devised. Human placenta was obtained under strict

aseptic precautions and from this a broth was made, treating the placenta just as the beef is treated in preparing the broth for common use. One per cent. peptone, one-half per cent. salt and five per cent. glycerin were added. The entire preparation of this culture medium was conducted as sterily as possible. In order not to destroy the complement of the human placenta, heat was applied only to 55° C. It was possible in most instances to obtain a sterile broth by means of filtration through a Berkefeld filter.

Two bovine strains of unknown virulence, one of them having been under artificial cultivation for many years, the other only recently isolated, were grown upon this culture medium for nine months, comprising eight generations. At the end of this period their virulence was found to be as marked as at the beginning, showing that in this respect they had not approached the human type. In fact, the only change that was noted was one to be anticipated from artificial cultivation of any nature, namely, a greater facility of growth in the case of the strain which had been the more recently isolated. As this experiment was very laborious and promised so little it was discontinued.

The question of the stability or variability of the types of tubercle bacilli has been discussed so frequently that I shall not review the testimony. However, in connection with this report, a few words upon this subject seem in place. From the outset it should be realized that this question has a practical as well as a theoretical aspect, and that the two need not be in absolute accord. The former considers whether it is in any way possible by natural or artificial means to convert one type of bacillus into another type; the latter whether the bacilli isolated from man and designated as human have been converted from an original bovine type. These two viewpoints must not be confused. It is true it has been demonstrated that under artificial cultivation strains may be greatly changed in their cultural and biological characteristics. Indeed, a culture isolated almost ten years ago from a cow, I recently found to simulate the human type both in luxuriance of growth and diminished virulence for rabbits. However such experiments clearly cannot be translated into practical evidence on this question. Again it may be advanced that experiments have been reported

showing a conversion of human into bovine bacilli by means of passage through cattle or of bovine into avian bacilli by means of passage through birds. However, even if we accept these interpretations, and forego the criticism that has been levelled at them by others, we find these experiments to be very few in number and far outweighed by the many others where no conversion of type was effected. When we consider the practical side of this question and draw our arguments from what we know of tubercle bacilli in man, the following should be borne in mind. Atypical types have been isolated by many investigators, strains neither typically human nor bovine. This intermediate group may in part be accounted for by attributing the variation to a change of environment, for example, of bovine bacilli in the new environment of human tissue. These atypical strains, it should be remembered, are exceptional; among three bovine and four human strains which I have isolated from man, none belongs to this category. Furthermore this deviation from type cannot be regarded as evidence of the possibility of a complete change of type. In this connection it is worthy of note that typical bovine bacilli have been isolated from calcareous lymph nodes of human beings; in such instances the bovine bacilli must have existed in the human tissues for years without undergoing transformation. It has never been shown that the older the tuberculous lesion the less the likelihood of isolating bovine bacilli from man. And yet this corollary should obtain if the bacilli are readily converted in the human tissues. The question may be approached from another point of view. It is well known that no case of primary pulmonary tuberculosis has been indisputably proved to have been incited by the bovine bacillus. These cases form the great mass of tuberculosis and have been studied more than all others. If this form of the disease is due in some instances to a bovine bacillus which has been transformed in the human body, we should at least occasionally meet with this type of the bacillus in the early stage of the disease when it has not yet been converted into the human type. Not only do we know that this is not the case, but we find that the intermediate types are rarely met with in the primary pulmonary cases. So that it would seem from a practical standpoint the question of transmutation of types

may well be disregarded and we can safely proceed to investigate the etiology of tuberculosis by means of differentiating the types of bacilli.

The two cases of cutaneous tuberculosis which I report claim some degree of interest because they constitute bovine infections occurring in adults. The fact that they remained localized should not be attributed to the low degree of virulence for man of the bovine bacillus, as it is well known that cutaneous affections due to the human type of bacillus, such as lupus or the "anatomical tubercle" contracted in the dissecting room, likewise do not tend to systematic invasion. Their main significance, however, lies in the fact that they furnish exceptional instances of bovine tubercle bacilli which have lived in the human tissues for years without acquiring characteristics of the human type. From this point of view they constitute evidence against the conversion in the human tissues of bovine bacilli into human tubercle bacilli. It would be interesting and of undoubted value if those who have access to similar long-standing cases of tuberculosis would make them the basis of bacteriological study.

ON THE DETERIORATION OF DIPHTHERIA ANTITOXIN

By EDWIN J. BANZHAF.

Lots in duplicate of native antitoxic sera, antitoxic citrated plasma and concentrated antitoxin globulin solution were taken. One lot of each was kept at ice-box temperature, varying from 4° to 7° C. The remaining lots at room temperature (22° to 26° C.).

On starting this work it was my intention to retest these lots every two months, but, after several retests, found it was too expensive an undertaking. I therefore lengthened the retest time to six months and later to once a year. The unit value of these lots of sera was determined very carefully with a toxin that was standardized every two months against a standard test serum furnished by the Hygienic Laboratory of the Public Health and Marine Hospital Service. The deteriorations of these various lots were as follows:

For the native antitoxic sera kept in ice-box, the average deterioration for one year was 14 per cent.; for two years the average was 22 per cent.; for three years, 24 per cent.

For the antitoxic citrated plasma, kept in ice-box, the deterioration was extremely low. The average for one year was 6 per cent.; for two years, 8 per cent.; for three years, 9 per cent.

The deterioration of potency with the concentrated antitoxic globulin solution, kept in ice-box for one year was 13 per cent.; for two years, 17 per cent.; for three years, 20 per cent.

The average deterioration of potency with the native antitoxic sera kept at room temperature for one year was 18 per cent.; for two years, 24 per cent.; for three years, 26 per cent.

For the antitoxic citrated plasma kept at room temperature the average deterioration for one year was 8 per cent.; for two years, 10 per cent.; for three years, 12 per cent.

For the antitoxic globulin solution kept at room temperature the average deterioration for one year was 16 per cent.; for two years, 20 per cent.; for three years, 23 per cent.

ON SOME ERRORS IN THE DETECTION OF GONOCOCCUS IN THE VAGINITIS OF CHILDREN

IRA VAN GIESON, M.D.

When a subject has been worn as threadbare as that of the microscopic detection of the gonococcus, it seems incredible that the simple procedure of collecting the exudate in vaginitis and spreading it on a slide for microscopic examination of the gonococcus could be so faulty as often to defeat the very purpose for which it is undertaken. Nevertheless, this is a fact. It has been supposed that the transference of the exudate from the vestibule or the vagina to the slide by means of a cotton swab, such as is used in taking cultures from the throat, would be thoroughly reliable; that is, that when the secretion is spread on the slide the presence or absence of the gonococcus would indicate the presence in or the absence of the organism from the vagina.

At the Willard Parker Hospital, in the scarlet fever, diphtheria and measles wards, and in the other hospitals of the Department of Health of the City of New York, we have for years taken this procedure at its face value supposing that it gave sufficient information in determining the question of the gonococcus in its important influence in our management of this prevalent, troublesome and persistent malady in little children. Now it turns out that the method is far from providing a certain means of detecting gonococcus, and frequently fails in cases without appreciable discharge. When a method is not fully reliable it is time for the substitution of one which is more certain and trustworthy. The new pipette method proposed here is so simple that it seems like making much of a small matter in according it any extended text. But the method deals with a troublesome and a rather common malady, and as it often happens that important phenomena are disclosed and handled with methods of trivial operation, no apology is needed for a seemingly inconsequential procedure if it helps us better to detect and to manage an intractable and easily transmissible disease.

Under any circumstance specific vaginitis in children is serious

From some standpoints, it is, perhaps, less so than in adults, in that the uterus and tubes are seldom involved; in other respects, however, it is far more serious because of the danger of ophthalmia. But everyone knows that in hospitals, especially in those for contagious diseases, the disease is particularly vicious because of its liability to spread through the wards unless the greatest vigilance is exercised. We have to be on guard constantly to catch the patient at the very inception of the disease, indeed, with the detection of the gonococcus to anticipate its appearance, and equal care is imperative in not sending a child home prematurely, to infect its companions.

When vaginitis is fully developed the gonococcus determination is hardly necessary to tell us what is going on. Nor when pus is pouring out of the vagina or urethra does anyone need a microscope to know what to do or to make a diagnosis, for he would only be making sure of what he already knew. To be sure, the microscope will give the secondary information as to whether the discharge is non-specific or the contrary. In such cases, when the aid of a microscope is to a certain extent a laboratory refinement, the swab device works very well, as indeed anything else which will get the exudate spread out uniformly on the slide. The method is excellent when the disease is so obvious that its application is hardly necessary.

This whole matter of the detection of the gonococcus, regarded from a practical standpoint, becomes of value in the very beginning and the declining stages of the discharge. These are the stages where the greatest danger of widespread infection exists for the simple reason that they are not heralded and placarded with the obtrusive cardinal manifestation of the malady, namely the discharge. Here we are absolutely dependent upon the microscope, and here, where it is important to single out vaginitis cases on admission to the hospital, to apprehend the beginning cases, to detect the gonococcus when the secretion is only faintly purulent or scanty and hidden in the vaginal fornices or otherwise not obvious, here it is that the device with the swabs sometimes leaves us in the lurch.

Whether the vaginal exudations without the gonococcus, the non-specific vaginitis cases are infectious or not is exceedingly difficult

to determine without inoculation experiments. From time to time we find a profuse non-gonorrhoeal vaginitis in which the leucocytes contained several small bacilli with tapering ends lying side by side. Apparently this form is infectious. Occasionally there are also non-specific cases in which there are intracellular cocci but distinctly smaller than the gonococcus. And rarely a discharge is met in which the leucocytes show no bacteria of any kind. The only organisms present are extracellular and seem to be merely the ordinary saprophytes of the healthy vagina. This form of vaginal discharge seems to be due to a simple catarrhal exudate accompanying at times measles and scarlet fever. The products of inflammation complicating the course of these exanthemata pass into the vagina and sweep out with them the ordinary vaginal bacterial flora.

The most sensible plan, I think, in the examination of these non-specific and nondescript discharges is, that while a negative report may be given as far as the gonococcus is concerned, in the question of isolating these children, we should be guided more by the general structural appearance of the exudate than the matter of the bacterial content, because we cannot tell whether these several bacteria associated with the non-specific cases are infectious or harmless. It is difficult to describe or lay down any set rule regarding the structural appearances of the smear which indicates a dangerous or suspicious discharge, but one learns from experience that a certain preponderance of leucocytes, or an exudate almost entirely purulent justifies the diagnosis from mere vaginal hypersecretion (from uncleanness, for instance), and the recommendation of isolation, even though the gonococcus is not present. A few leucocytes scattered among a large content of vaginal epithelial scales, although the smears may be laden with various kinds of bacteria, has no special significance. In the routine microscopic vaginal examinations at the Department of Health hospitals (comprising at times several hundred children) I have followed this plan of dealing with the non-specific or indifferent discharges largely according to the structural appearance of the exudate, reporting, for instance, "Gonococcus absent. recommend isolation and observation for several days

from largely purulent character of discharge and slight relative content of desquamated vaginal epithelium."

Considering the volume of vaginal discharge both benign and infectious, and the catarrhal conditions in measles and scarlet fever that we have to contend with, this policy has worked out very well. It places one on the safe side with many children having discharges which are not gonorrhoeal. If the discharge is benign no harm is done to the child in isolation, and, if not gonorrhoeal, but nevertheless infectious, a menace to the ward is removed. From what has been said it is apparent that adherence to a rigid rule in the absence or presence of gonococcus in the determination of this question of vaginal discharges may bring about diagnostic blunders in two ways. The observer might find organisms indistinguishable from gonococci in a vaginal secretion without the structural earmarks of a true vaginal inflammation and so condemn a child with mere profuse secretion to the vaginitis ward, and, on the other hand, he might in reporting the absence of the gonococcus leave an infectious child in the ward. Some judgment must be exercised independent of, or at least in conjunction with, the question of the morphology of bacteria in these secretions. The structural character of the secretions must be taken into consideration, and at times this is just as important a factor as the bacterial determination.

The reason for the frequent failure of the dry swab method is quite simple. Unless the discharge is profuse, the exudate on the absorbent cotton is caught in its interstices, and the result is that much of what we wish to have on the slide is retained by the cotton. Not infrequently, in thin, serous and scanty discharges, nothing but fluid is expressed from the swab to the slide, while abundant gonococci remain in the leucocytes sticking tenaciously within the meshes of the cotton fibres. In attempting to release the exudate from the preparation of the smear, the swab is rubbed and pressed about on the slide; the cellular contents may be torn off the cotton fibres with considerable mechanical injury so that the structure of the material on the slide, especially the leucocyte, is highly distorted and most unfavorable for the detection of gonococcus. Quite frequently, also, it is difficult to

spread the exudate evenly and uniformly on the slides with the swab; thus the cells are heaped on top of each other and conceal the gonococci. Occasionally the narrow vaginal orifice in children wipes the surface layer of the exudate off the swab as it is being removed.

The intact leucocyte cell body is the *sine qua non* in the diagnosis of the gonococcus. Without this we lose the foothold of certainty. This is especially true of the vaginal secretion, which brings in difficulties not encountered in discharges from the male urethra. In the vaginal discharge we cannot take the free cocci into account, because, while the male urethral exudate contains as a rule no adventitious bacteria and only those of a specific or non-specific urethritis, the vaginal discharge and the healthy vagina may be contaminated with a great number of various bacteria, among them cocci, which cannot be distinguished morphologically from gonococci.

If one were to make the diagnosis on the presence of free gonococci-like organism, many children, healthy as far as vaginitis is concerned, would be wrongly put into the vaginitis ward. These smears with distorted pus cells are, therefore, misleading and often quite worthless. It is truly astonishing to discover that hundreds and hundreds of gonococci may be lost or concealed in the smears from these cotton swabs or retained on the cotton. It should not be understood that the deformity of the leucocytes is characteristic of all swab smears. It occurs extensively, however, in quite a large proportion of them when the secretion is very thin or viscous, and especially when the swab is allowed to dry in the air momentarily or through a conjunction of both factors. Occasionally the volume and consistence of the exudate is such that the film is in good condition and offers the proper conditions of accurate diagnosis.

Besides these drawbacks, it is questionable whether the swab device is wholly harmless. No one would think of using a similar procedure in the male urethra, because it would denude the epithelial layer and damage the lining membrane. The introduction of a dry cotton swab into an inflamed and sensitive vagina, twisting it about to collect the exudate must injure the lining walls if only to a slight extent. And since, as we are told, the deeper layers of the epithelium and the mem-

brane on which it rests act as barriers against invasion of the gonococcus into the circulation or the lymph spaces and its propagation to remoter parts, this aspect of the procedure is hardly a negligible factor. In the final and healing stages of vaginitis it is also important not to interfere with the restitution of the epithelium. If swabs must be used it would be better to wet them before they are applied; the smears are better and their introduction easier.

A very much better way to obtain the exudate is to collect it in glass tubes so that one can see what the content of the vagina is, and incidentally not lose it in transferring it to the slide. For this purpose ordinary medicine droppers are the most convenient; they are about the length of the average child's vagina and manipulation of the rubber bulb collects the secretion. Occasionally the sharp cutting edge of the dropper needs rounding off in the flame. The tubes, of course, may be sterilized and used over again, kept in bichloride solution before being applied, and the children are hardly aware of their introduction, which is more than can be said of the swab. Sometimes the pus is so scanty that instead of filling the lumen of the tube it sticks to the sides of its walls and cannot be transferred to the slide. In such instances it is well to fill the tube with a drop or two of water, or better, a 1-5000 bichloride solution before introducing into the vagina and by compressing and expanding the bulb make an emulsion of the exudate. The advantage of this is that the whole vaginal contents is obtained where the secretion is scanty, the bichloride fixes the cellular elements at the same time, and they are in perfect form when dried on the slide. Salt solution is less preferable, for it seems to contract the leucocytes. Exceptionally in very young children, or where the vaginal entrance is very small, it is necessary to draw out the tubes to a finer calibre, although when partly filled with the bichloride solution they need only to be inserted into the ostium and the exudate washed out into their lumen.

This method is perfectly adapted to the study of structural cellular details of the exudate, which under the generic title of the "cyto-diagnosis" of urethral and vaginal discharges came into prominence some two or three years ago. Up to this time observers had largely restricted their study of these exudates merely to the detection of the gono-

cocci. The advance of the technique of staining blood and differentiation of the various white blood cells and their constituent granules suggested a similar study in urethral and vaginal discharges. Pappenheim, Janowski, Joseph, Palano, Posner and several other observers took up the subject and tried to turn the study to practical account in diagnostic and prognostic value. They found various types of leucocytes, such as mast-cells, eosinophiles, small and large lymphocytes, globular nucleated polymorpho-nuclear leucocytes, vacuolated and other types of degenerated cells, and attempted to show that the prevalence or absence of one or another type of these various cells corresponded to some particular phase of the disease and hence was of diagnostic value. At the request of my friend, the late Dr. Robert W. Taylor, I went over this subject, not only in the male urethral inflammations in various stages, but also in vaginitis, using the various methods of blood smear technique, and feel quite sure that while the subject may be exceedingly interesting from a theoretical standpoint, it does not work out at all in practical diagnostic value, for the results are too irregular and contradictory. Thus the eosinophiles are liable to occur in the early stages, but they occur at times equally well in the late and chronic stages and so on with various other constituents of the exudate. There are but two signs indicative of particular phases in gonorrhoea, and even these are rather vague and indefinite, namely, the presence of red blood cells, indicating a beginning stage of gonorrhoea and the appearance of considerable epithelium, mucus and the decrease of the leucocytes, showing that the process is nearing its end stages, which is a good deal like saying that the shower is over when the rain drops cease falling.

In contrasting these two methods, at Dr. Park's suggestion I followed several cases in the vaginitis wards, systematically controlling the swab smears with tube method; sometimes immediately afterwards, sometimes twenty-four hours later. Here are the results as regards the absence or presence of the gonococcus indicated respectively by the minus and plus signs.

TABLE 1

	Feb. 9, '09 Dry Swab Smears	Feb. 10, '09 Control Medicine Dropper
Clara N.....	—	+
Dora A.....	—	+
Dora K.....	+	+
Annie K.....	+	+
Sadie D.....	+	+
Mary A.....	+	+
Lucia.....	+	+
Katie M.....	—	+
Caroline.....	+	+

TABLE 2

	Feb. 12, '09. Dry Swabs.	Moist Swabs.	Feb. 13, '09. Medicine Droppers.
Rosie G.....	+	—	+
Sadie L.....	+	+	+
Helen A.....	—	+	+
Annie O.....	+	+	+
Anna P.....	—	—	+
Ella S.....	+	+	+
Fannie S.....	+	+	+
Mamie G.....	—	—	—

These tables need no comment. It will be seen that in many instances where the swab method has given a negative decision the gonococci are nevertheless present, as shown in the smears from the medicine droppers. In table No. 1 the swabs have worked fairly well, giving positive results in 6 out of 9 cases.

In table No. 2 the suggestion of moistening the swab was carried out, giving slightly better results. These children ranged between the ages of one to six or eight years, and most of the results in each method were controlled by staining with Gram's method. It is the custom at these hospitals to examine all of the children twice a week for gonococci and all cases on admission. I have tried not to fall into the rather natural tendency of accentuating the value of a measure by exagger-

ating the defects of what it is designed to correct, for the swab smears have always been taken by the interne physicians of the hospitals,* and represent, I think, on an average the results obtained in hospitals in general when the method is used. Perhaps the swabs might have been taken more carefully, especially if one had in mind their imperfections, and the tables are not wholly fair, but the procedure is certainly inferior to, and less reliable than that of the tubes. This is especially to be emphasized *when the gonococci are very few in number, for they may be lost entirely in the swabs even when moistened.*

A striking instance of the value of the new method is the case of Katie L., 5 years old, admitted with scarlet fever. The swab smears were negative. While the child was still on the examination table I was astonished to find fully half of the medicine dropper tube (plus a two or three drops weak bichloride solution) filled with pus hidden in the vaginal fornices and which was reeking with gonococci. There was hardly any external evidence of the discharge and this child, if we had relied on the swab method result, would have been sent into the ward to infect others of the inmates.

Another interesting case is that of Ellen F., but 3 years old, who had been in the diphtheria ward some three weeks. This ward on the sixth floor of the Willard Parker Hospital contained 24 children. Ellen had no discharge, no signs of vaginitis, no gonococci on admission or any other time as far as could be determined, when suddenly, on March 27, 1909, a discharge developed. The swab method failed but the medicine dropper disclosed abundant pus cells with gonococci. No other child had vaginitis in the ward, and a question of great interest arises: How did Ellen develop a specific vaginitis three weeks after admission when the examination was negative and when there were no cases in this ward to infect her?

These cases are not very rare and their consideration, which is beside the issues of this paper, seems very important not only for study of gonorrhoeal infection in particular but for the role of pathogenic bacteria in general. Apparently the explanation in consonance with the

* As far as I can learn the swab method is quite universal in the various hospitals in New York City which contain any considerable number of children.

theories of the present day is that such children are gonococcus carriers; that such gonococci do not provoke any pathogenic action in these particular children until some complicating congestion or light catarrh, incident to diphtheria and certain of the exanthemata provide the suitable opportunity. We hope, during the ensuing year, to see if, by an accurate method of examination of admission cases, this can be substantiated; to see whether these children belong to the class of bacteria carriers, as we have come to know them in connection with typhoid fever, diphtheria, etc.

This investigation will not only require the washing out of the vaginal contents into the bichloride tubes, but probably also depositing the cells, before transferring to the slides, by the centrifugal machine, since the scanty collection of cells will be dispersed in a considerable volume of fluid.

A third instance of the accuracy of the tube method worth mentioning, perhaps, is that of a declining discharge of a girl of fifteen years. An unequivocal decision of the question of the gonococcus was highly important because medico-legal consideration involving the matter of criminal assault entered into the case. The physicians to whom the gonococcus determination was referred were unable to settle this question one way or the other, because smears prepared in the conventional way from absorbent cotton swabs showed such mutilation or destruction of the leucocytic cell bodies that no intracellular gonococci were found, and they declined—quite properly—to make a diagnosis on the presence of free “coffee bean” diplococci amid the variegated vaginal bacterial flora.

As a matter of fact gonococci were present but exceedingly few in number, as was disclosed by flushing out the vaginal fornices with a tube filled with a few drops 1-1000 bichloride solution. These smears showed the classical undoubtable picture groups of gonococci within the bodies of perfectly preserved leucocytes.

A tube similar to the medicine dropper might also be introduced into the meatus of the male urethra in certain cases of very slight discharge and the canal flushed out to collect the exudate for gonococcus detection.

The swab procedure is equally bad in determining the nature of inflammation or discharges from the conjunctiva. This is so very important that it needs no elaboration. Among other forms we are constantly meeting with simple catarrhal conjunctivitis in measles and scarlet fever, diphtheric conjunctivitis, and at times an inflammation accompanied by the pneumococcus, Morax, Axenfeld or Koch-weeks bacillus, but we never can be sure without the microscope, whether the case may not be one of gonorrhoeal ophthalmia.

I know of at least one case of very early gonorrhoeal ophthalmia in which the swab method utterly failed to show the very few gonococci present disclosed by the tube procedure. Exudates from the conjunctiva should be allowed to flow into capillary tubes. If such exudates are thick and viscous there may be drawn up into the tube a piece of attached capillary rubber tubing and expressed on the slide and emulsified with weak bichloride solution to make the smear spread thin and uniformly. The same may be said of the examination of cases of otitis and discharges from the ear, sinuses, etc.

A trivial suggestion in the staining of the smears, although very likely gratuitous, may be of service to those having occasion to make these determinations in large numbers. The fixing of the smear may be discarded. The film can be flooded with almost any of the polychrome solutions, washed in water and examined while wet under the cover glass or afterwards dried for permanent keeping. In fact, the washing in water may be omitted and a drop or two of the polychrome solution, if not too opaque, may be allowed to flow in a capillary sheet over the film underneath the cover glass and the examination made directly. An advantage of this is, besides saving time, that the gonococci appear to swell slightly and approach more nearly their size and shape in the living condition, which is of service in comparing them with a gonococcus-like organism somewhat smaller in size.

TOXIN-ANTITOXIN MIXTURES AS IMMUNIZING AGENTS

BY

WILLIAM H. PARK, M.D., and L. W. FAMULENER, M.D.

Preliminary Note

Ehrlich early suggested that injections of diphtheria toxin partially neutralized by antitoxin would probably produce active immunity. This was demonstrated by Wernicke, Dreyer and Madsen, Morgenroth, ourselves and others. Smith, in a recent article, suggested the possible use of such mixtures in the immunization of children.

The possibility of such a practical application has suggested to us some experiments with especial reference to the safety and effectiveness of the injections.

The proportion of toxin to antitoxin in the mixture necessary to produce immunization was investigated by us in horses.

In May, 1903, we reported some experiments in which one set of horses were injected with mixtures containing toxin 0.66 of L_{\dagger} dose for each unit of antitoxin and another with four times this proportion of antitoxin. Three large injections at five-day intervals each of 100,000 fatal doses of toxin produced in the first series with .66 L_{\dagger} an average of 150 units per cc. of serum, in the second with .16 L_{\dagger} an average of only 3 units.

Smith injected three guinea pigs with 2 units plus 1.5 L_{\dagger} dose, 2 units plus 1.3 L_{\dagger} dose, and 2 units plus 1 L_{\dagger} dose respectively. The litters born from the first and second animals showed marked immunity at the end of 8 months. The litter from the third animal showed slight immunity at 3 months and none at 6 months.

These experiments indicate that while even a proportion of 6 units of antitoxin to 1 L_{\dagger} of toxin produces slight immunity, the toxin must be in proportion of at least 1 L_{\dagger} dose to 2 units to cause the production of any considerable amount of antitoxic immunity.

This brings us to the question of the safety of such mixtures. The work of Morgenroth suggests that mixtures which are toxic for one species are toxic for all. There is a difference, however, among ani-

mals as to the amount of natural immunity, therefore, of the amount of toxin required to produce the development of serious symptoms or death. Even if all guinea pigs lived, therefore, there would still be a slight uncertainty in infants.

In a series of tests we found that the least toxin which was necessary to give lasting immunity was not quite harmless in guinea pigs. Thus of four guinea pigs receiving a mixture of 1 unit plus 6/10 L_{\dagger} dose of toxin the two larger remained permanently well, while the two smaller finally died of paralysis. Four guinea pigs receiving one-half the quantity of the same mixture all remained alive. Two other series receiving still larger quantities of the same mixture acted as the first lot. Some of these guinea pigs after two months received later two fatal doses of toxin without serious poisoning.

It is interesting to note that three of these animals later received repeated injections of toxin in increasing amounts, until finally 6,000 fatal doses were given in one injection. The blood of the animals at this time contained about from 25 to 30 antitoxin units per c.c. These experiments suggest that it is hardly safe to attempt to immunize children with toxin-antitoxin mixtures since when a degree of saturation with antitoxin is used that is absolutely safe the amount of immunity produced is small. This immunity is much less for the first two weeks, than when the same amount of antitoxin is injected without the toxin. It is possible that the toxin used in these experiments, which was produced by our culture No. 8, may have more tendency to promote late paralysis than that from other cultures.

THE COMPLEMENT BINDING TEST IN RABIES.

JANE L. BERRY, M.D., ASSISTED BY MISS A. MANN.

The work of A. Wasserman and his collaborators in the application of the complement binding test of Bordet and Gengou to the diagnosis of syphilis and other diseases has been followed, as is well known, by a long series of investigations, and many observers have confirmed the findings of Wasserman. Positive reactions are said to have been found in typhoid, tuberculosis, meningitis and many other diseased conditions. The most striking results have been those obtained in syphilitic infection.

Many views have been expressed with regard to the nature of the reaction. The original claim that specific antigens as well as anti-bodies could be demonstrated in this manner was soon abandoned, since it was proved that the test was obtained not only with specific, but also with normal extracts. The fact that alcoholic extracts were found to be effective showed the active substance to be alcohol soluble or lipoid in nature. Porges and Meier, working under Wassermann's direction, found that pure lecithin was able to bind complement with syphilitic sera, and it was evident later that a large number of other lipoids acted as well or even better than lecithin. Meantime, Landsteiner and Stan-kovic had obtained similar results with numerous organically suspended and colloid dissolved substances. Axamit, working with bacterial extracts, found that these alone bind complement, while Uhlenhuth obtained binding with a long list of the most unrelated and diverse materials.

None of these results have been held to affect the value of the test in the opinion of its supporters, since it is their claim that complement binding can be obtained only by the action of specific, never of normal, sera, or, if the contrary is ever true, it is said to be so rare that it can be practically disregarded. These views have not met with universal acceptance, however, since opposite results have been found by several prominent investigators.

Ranzi obtained binding of complement with normal sera alone,

with extracts of tumors alone, and with tumor extracts combined with normal sera. Weil and Braun found binding to the same extent with normal as with specific sera together with tumor extracts. They considered the active agent to be not specific in nature, regarding it as probably a substance derived from degenerative cell changes in various diseased conditions. Much, testing sera from 25 cases of scarlet fever with syphilitic liver extract, found binding in 10. He obtained a similar reaction in many other diseased conditions and also with normal sera, and together with Eichelberg regarded the test as without diagnostic value.

In 1907 Heller and Tomarkin¹ sought to demonstrate, by means of the complement binding test, the existence of specific antibodies against rabies virus in the serum of animals immunized against rabies. They used the sera of rabbits inoculated subcutaneously on twenty-four consecutive days with extract of fixed virus rabbit cords made from two to eight days after death, testing the sera two to three weeks after the last injection. As test fluid they used the expressed juice of rabbit's brains obtained under pressure of 350 atmospheres, the animals having died from fixed or street virus, which were tested separately. All customary controls were used, and the experiments frequently repeated with varying amounts, but the results were negative as to diagnostic value of test in rabies. Occasionally increasing inhibition of haemolysis was obtained by the use of increasing amounts of specific immune juice and immune sera, but the same result was found upon using immune sera with expressed juice of normal brains, or normal sera together with specific or normal expressed juices.

Friedberger,² from a theoretical standpoint, thought this test especially well adapted for rabies. He used the serum of a horse inoculated with extract from a fixed virus rabbit brain, the serum of a rabbit immunized to horse blood, guinea pig complement, and the customary controls. He found a small amount of binding with rabies serum, but no stronger than with serum of normal horse and came to the same conclusion as Heller and Tomarkin, that no positive conclusions could be based upon this test in rabies.

A third report of complement binding tests with rabies was pub-

lished after the completion of the present experiments. Baroni and his associates used three sera from animals immunized to rabies. Two were from rabbits inoculated with fixed virus rabbit cord emulsion, the first rabbit having died after six subcutaneous inoculations followed by one into the anterior chamber of the eye; the second having resisted a similar eye inoculation after one previous intravenous injection. The third serum, from Marie, was from a sheep given multiple subcutaneous injections of fixed virus at the Pasteur Institute. The three sera were tested against spleen and brain of rabbits immunized as above, with fresh guinea pig complement, according to the classical method, but, notwithstanding numerous trials no binding of complement could be demonstrated, haemolysis being always identical with that in controls of normal rabbit serum.

At the suggestion of Dr. Williams the writer undertook the present series of experiments with rabies.

The rabies material tested consisted of extracts of the brains of fixed virus rabbits, the rabbits being those furnishing the cords used in the treatment of rabies patients. The fresh brain material was crushed in a sterile tube, mixed in proportion of 1:4 with normal salt solution containing 0.5 per cent. carbolic acid, agitated at room temperature for 24 hours, and centrifuged for from one to two hours, the supernatant fluid being then drawn off into sterile flasks and placed in the ice box till needed.

Extracts were made in the same manner from the brains of normal rabbits.

A series of normal rabbits were inoculated subcutaneously with rabies material; Rabbit A, for the first few inoculations, with the above fixed virus extracts; later, this rabbit and all others of the series were injected at intervals of ten days with an emulsion of fresh fixed virus brain in salt solution, beginning with $\frac{1}{4}$ c.c., and increasing 0.2 with each treatment up to the amount of 2.5 c.c. at the last inoculation. After the fourth treatment the serum of these rabbits was used with the fixed virus brain extract for the complement binding test. Control tests were made with the serum of normal rabbits and of normal guinea

pigs, and both rabies and normal sera were tested against the normal brain extract by the same method.

The blood cells used were those of the horse washed four times with salt solution, a series of rabbits having been immunized against horse blood by subcutaneous inoculations of 10 c.c. of washed horse blood cells at ten day intervals. One of these rabbits developed sufficient hemolysin after the third inoculation to dissolve 1 c.c. of a 5 per cent. suspension of horse blood cells with 1-1000 c.c. of serum. This amount gradually decreased until 1-200 c.c. of serum was required for the same amount of blood at the time of the last experiment. The amount of hemolytic serum used in each test was twice that needed for blood solution, the quantity having been ascertained by preliminary tests.

All sera were inactivated by heating at 56° for one-half hour.

Complement was furnished by fresh normal guinea pig serum.

The various substances were first diluted with normal salt solution to such strength that 1 c.c. of each could be taken for the test, making 5 c. c. in all. When one or more substances were omitted, the tubes were filled to the same level with normal salt solution. Thus diluted the fixed virus or normal extract, and fixed virus or normal serum, were measured into sterile tubes, the complement added and the tubes placed in the incubator for one hour, when the blood cells and hemolytic serum were added, the tubes returned to the incubator for two hours, then placed in the ice-box. The next morning the tubes were thoroughly shaken and again placed in the ice-box until the following morning, when the final reading was made.

The experiments were many times repeated, using sera from four fixed virus rabbits, and from two normal rabbits, together with extracts from four fixed virus rabbits and from four normal rabbits, in varying amounts and combinations, together with controls as shown in table. In this way over fifty tests were made, showing in all the same result—complete or practically complete inhibition of hemolysis. No difference whatever could be perceived between the action of normal and of fixed virus serum, except that in one series a slightly stronger binding power was shown by the normal than by the fixed virus serum. In one case the normal serum and normal brain extract were both obtained from

The following table is representative of results obtained.

TABLE I.

<i>Sera.</i>			<i>Extracts.</i>			
Fixed Virus Rabbit.			Fixed Virus Rabbit Brain.			
Normal Rabbit.			Normal Rabbit Brain.			
Serum	Extract		Com- ple- ment 1:10	Haem Ambo- cept 1:100	Blood 5%	Result
F.V. Rab., .2 c.c.	F.V. Ext., .2 c.c.	1 c.c.	1 c.c.	1 c.c.	1 c.c.	Practically Comp. Inhibition
" " .1	" " .2	"	"	"	"	Nearly Complete Inhibition
" " .01	" " .2	"	"	"	"	Complete Haemolysis.
" " .006	" " .2	"	"	"	"	" "
" " .005	" " .2	"	"	"	"	" "
" " .2	Norm. Ext. .2	"	"	"	"	Practically Comp. Inhibition
" " .1	" " .2	"	"	"	"	Nearly Comp. Inhibition
" " .01	" " .2	"	"	"	"	Complete Haemolysis
" " .006	" " .2	"	"	"	"	" "
" " .005	" " .2	"	"	"	"	" "
Norm. Rab., .2	F.V. Ext., .2	"	"	"	"	Practically Comp. Inhibition
" " .1	" " .2	"	"	"	"	" " "
" " .01	" " .2	"	"	"	"	Complete Haemolysis
" " .006	" " .2	"	"	"	"	" "
" " .005	" " .2	"	"	"	"	" "
" " .2	Norm. Ext., .2	"	"	"	"	Practically Comp. Inhibition
" " .1	" " .2	"	"	"	"	" " "
" " .01	" " .2	"	"	"	"	Complete Haemolysis
" " .006	" " .2	"	"	"	"	" "
" " .005	" " .2	"	"	"	"	" "
F.V. Rab., .2	"	"	"	"	" "
" " .4	"	"	"	"	" "
Norm. Rab., .2	"	"	"	"	" "
" " .4	"	"	"	"	" "
.....	F.V. Ext., .2	"	"	"	"	Complete Haemolysis.
.....	" " .4	"	"	"	"	Occasional Sl. Inhibition
.....	"	"	"	"	Complete Haemolysis.
.....	Norm. Ext., .2	"	"	"	"	Occasional Sl. Inhibition
.....	" " .4	"	"	"	"	Complete Haemolysis.
.....	"	"	"	"	Occasional Sl. Inhibition
F.V. Rab., .4	"	"	No Haemolysis
Norm. Rab., .4	"	"	" "
.....	F.V. Ext., .4	"	"	" "
.....	Norm. Ext., .4	"	"	" "
.....	1 c.c.	"	"	" "
.....	1 c.c.	"	"	Sl. Tr. Hemolysis
System Control			"	"	"	Complete Haemolysis

the same rabbit, but the result here was identical with that in all the other tests. The slight haemolytic action of normal guinea pig serum upon horse blood corpuscles, frequently noted, was not sufficiently marked to interfere with the definite comparison of results, the same being true of the slight inhibitory action sometimes seen in both fixed virus and normal extracts. Inhibition was always complete as to the action of the haemolytic serum, with invariable full hemolysis in haemolytic system controls.

These experiments agree with those of former investigators in showing the absence of any specificity for rabies in the complement binding test.

That the normal sera should have shown an equal inhibitory power to that of the fixed virus sera remains unexplained, but it is felt that errors of technique and merely accidental results are ruled out by the number of experiments made, and by the confirmatory action of the numerous controls.

The complete haemolysis obtained with all amounts of serum below 0.1 c.c. would seem to exclude precipitins here as the probable cause of complement binding.

To the above a few additional tests may be added. Serum from a normal rabbit, 0.2 and 0.4 cubic centimeters, has been brought together with extract of normal rabbit brain (from same individual), 0.2 cubic centimeter, with hemolytic amboceptor from rabbit, with fresh guinea pig complement and with sheep corpuscles. Result:—partial hemolysis (0.8) with smaller dose of serum; slight hemolysis (0.2) with larger dose. The experiment repeated, substituting extract of brain of rabbit with rabies instead of normal extract, shows slight hemolysis (0.3) with smaller dose and slight trace of hemolysis (0.1) with larger dose. The same experiments in which sera from two normal guinea pigs have been tested together in place of rabbit serum, shows complete hemolysis. There is no trace of inhibition with either extract. These results with rabbit's serum constitute the first instance in this series of tests in which any inferior binding power has been shown by normal rabbit's serum with normal brain extract, as compared with brain extracts of rabbits with rabies. In this instance an

additional factor has to be taken into account, namely, the normal serum and normal brain extract have been obtained from the same animal.

An extensive bibliography upon the complement binding test is given by Fleischmann⁴ and by Noguchi⁵. No other references are given here except those relating to the test with rabies.

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THE INFLUENCE OF CHLORAL HYDRATE ON SERUM ANAPHYLAXIS.*

EDWIN J. BANZHAF AND L. W. FAMULENER.

In a preliminary communication read before the Society for Experimental Biology and Medicine in February, 1908, we reported the following:

"We found that by injecting a solution of chloral hydrate which was just sufficient in strength to produce hypnosis, fully 75 per cent. of all serum-sensitized guinea pigs were completely protected from a second injection of serum into the peritoneal cavity, whereas 90 per cent. of controls died. We believed that with improved technique in the dosage of chloral hydrate it would be possible to protect 90 per cent. of all fully sensitized guinea pigs. By "fully sensitized" we meant that at least three weeks or a month's time should have elapsed before the second injection of serum into guinea pigs which had received horse serum alone (1-1000 to 1-500 c. c.), at least seven or eight weeks should have elapsed before the second injection. By allowing the above interval of time to elapse, over 90 per cent. of our controls died within an hour, most of them within 20 minutes.

"We have found that the dose of chloral hydrate per gram weight of the animal was not a simple ratio; no fixed amount could be stated, much depending upon the individual idiosyncrasy of the animal. Approximately, 75 milligrams of the drug to a 250-gram guinea pig, and 100 milligrams to a 300-gram guinea pig, produce the degree of hypnosis desired.

"We used a fresh 10 per cent. solution of chloral hydrate, carefully measured out the required amount into a small sterile beaker, and added an equal amount of sterile water. This diluted solution was injected into the muscles of the thigh of the animal, half into one leg and half into the other. After 20 to 30 minutes the needle was inserted into the peritoneal cavity. This caused muscular twitching and slight movement of the head. This indicated the proper degree of hypnosis. The injection of 5 c.c. serum was then given and the animal kept in a warm room. No symptoms appeared and the sleep was undisturbed. After 2½ to 3½ hours had elapsed the animal slowly recovered from the effect of the drug. No symptoms or ill effects have been observed in any of the animals. Observations have been followed for over two weeks after treatment.

"The animal, after the effects of the drug have disappeared, will react with characteristic symptoms of anaphylaxis if given a third injection of the serum. We injected only 24, 48 and 72 hours after recovery from the effects of the drug.

"If the dose of chloral hydrate has not been sufficient, the insertion of the needle into the peritoneal cavity will cause marked muscular movements, raising of the head and an attempt to regain its feet. Under these conditions, if the serum is injected the animal will die of anaphylaxis.

"On the other hand, if the animal shows no muscular twitchings whatever

*Read before the American Society of Biological Chemists, Baltimore, December, 1908.

the dose of chloral has probably been too large. We wish to emphasize the fact that great care must be used not to overdose the sensitized guinea pig with chloral hydrate, although a sensitized as well as a normal guinea pig will recover from a large dose—considerably more than the amount mentioned above. Apparently, the combined effects of the drug and the serum in a sensitized animal produces a deeper hypnosis than the drug when given alone.”

Further work, following along the lines of the above preliminary report, has fully confirmed our earlier observations.

We realized that for our work it was essential to determine the best method for sensitizing the guinea pigs so that on receiving the second injection of horse serum intraperitoneally the animals would surely die from anaphylaxis. We, therefore, injected several series of guinea pigs subcutaneously with from 0.01 c.c to 0.001 c.c. of horse serum. After three to six weeks we found that in none of the series did 100 per cent deaths occur when the animals were injected with 5 c.c. horse serum intraperitoneally. In our later work we have used only those guinea pigs which had survived the routine testing of antitoxin. After a lapse of three weeks or longer, these animals, in our experience, succumb almost without exception to the second injection of 5 c.c. horse serum intraperitoneally.

In this later work, we have only given the second injection of serum to those sensitized guinea pigs which showed favorable hypnosis, indicated by only slight muscular twitchings when the needle is inserted into the peritoneal cavity. If muscular movements are marked we do not inject. In this case we allow the animal to recover from the effects of the drug, and next day increase the dose about $1/5$. This usually brings about the optimum hypnosis in about twenty-five to thirty minutes. In following this method we have frequently protected 100 per cent., while 100 per cent. of the controls died.

In our work thus far it appears as though the protection chloral hydrate affords a sensitized guinea pig depends on the degree of sensitization. If we accept the hypothesis of Vaughan, this may be expressed as the amount of ferment or zymogen present in the sensitized guinea pig. Its supposed degree of activity or power in freeing or splitting off definite quantities of a poisonous substance from the serum

given in the second injection accounting for the symptoms of anaphylaxis.

This appears to be the case in Table I. The eight guinea pigs were well under the influence of the drug, when the second injection was given. Numbers 393, 702 and 146 died in 15, 30 and 20 minutes respectively, with typical symptoms of anaphylaxis, the heart continuing to beat after respiration ceased.

(For Table I, see next page.)

TABLE I
INTRAMUSCULAR INJECTION OF CHLORAL HYDRATE—FOLLOWED BY 5 c.c.
NORMAL HORSE SERUM

G. P. No.	Wgt.	Previous Treatment	Interval in Days	Chloral Hydrate in mg.	Second Injection Intraperitoneally	Symptoms	Results
330	310	0.56 c.c. toxin + 1/500 c.c. antitoxic horse serum	42	125	5 c.c. normal horse serum	None	Recovered
393	265	"	42	90	"	Typical	Death within 15 min. Recovered
355	260	0.56 c.c. toxin + 1/300 c.c. antitoxic horse serum	28	90	"	None	Recovered
376	260	"	28	85	"	"	"
358	260	0.56 c.c. toxin + 1/350 c.c. antitoxic horse serum	32	85	"	"	"
363	280	"	32	100	"	"	"
702	395	0.56 c.c. toxin + 1/300 c.c. antitoxic horse serum	62	180	"	Typical	Death within 30 min.
146	335	"	62	150	"	"	Death within 20 min.
313	395	0.56 c.c. toxin + 1/500 c.c. antitoxic horse serum	42		"	"	Death within 18 min.
372	260	"	42		"	"	Death within 25 min.
595	240	0.56 c.c. toxin + 1/200 c.c. antitoxic horse serum	28		"	"	Death within 12 min.
696	240	"	28		"	"	Death within 17 min.
513	230	0.56 c.c. toxin + 1/300 c.c. antitoxic horse serum	33		"	"	Death within 12 min.
347	260	"	33		"	"	Death within 33 min.

Many series of sensitized guinea pigs were given chloral hydrate intramuscularly followed by the second injection of horse serum, intraperitoneally and in almost all cases one or two guinea pigs of each series would die with typical symptoms of anaphylaxis, even though the degree of hypnosis seemed sufficient. This leads us to believe that in such guinea pigs more poison was elaborated or split off from the serum, in a given time, than in those that were protected; for example, say, if just a single fatal dose of poison is split off the chloral will protect; but, if two or more fatal doses are split off, the chloral cannot protect the animal. A quantitative relationship may exist.

In table II the controls died promptly, while those which received the chloral were protected.

Guinea pigs No. 60 and 202 had received 180 and 160 milligrams chloral, respectively, the day before. They then weighed 410 and 370 grams. This amount, however, proved insufficient to produce the desired hypnosis. They were allowed to recover, and the following day weighed 370 and 335 grams respectively. They were again given the same amount of the drug, 180 and 160 milligrams. In twenty-five minutes favorable hypnosis was produced. The five cubic centimeters of horse serum were then injected. The following day these two guinea pigs were chloroformed and autopsied. No definite lesions were found.

Guinea pigs Nos. 240, 777, and 748, three days later, again received five cubic centimeters horse serum intraperitoneally; slight but definite symptoms appeared in the first two animals after about fifteen min-

TABLE II
INTRAMUSCULAR INJECTION OF CHLORAL HYDRATE—FOLLOWED BY 5 C.C.
NORMAL HORSE SERUM

G. P. No.	Wgt.	Previous Treatment	Interval in Days	Chloral Hydrate	Second Injection Intraperitoneally	Symptoms	Results
60	370	0.56 c.c. toxin + 1/350 c.c. antitoxic horse serum	85	180	5 c.c. normal horse serum	Slight twitching of ears	Recovered
202	335	0.56 c.c. toxin + 1/750 c.c. antitoxic horse serum	82	160	"	None	"
240	370	0.56 c.c. toxin + 1/400 c.c. antitoxic horse serum	74	170	"	"	"
777	325	0.56 c.c. toxin + 1/800 c.c. antitoxic horse serum	68	130	"	Slight twitching of ears	"
748	345	0.56 c.c. toxin + 1/250 c.c. antitoxic horse serum	68	155	"	"	"
438	375	0.56 c.c. toxin + 1/200 c.c. antitoxic horse serum	85		"	Typical	Death within 20 min.
143	360	0.56 c.c. toxin + 1/300 c.c. antitoxic horse serum	82		"	"	Death within 13 min.
251	395	0.56 c.c. toxin + 1/250 c.c. antitoxic horse serum	81		"	"	Death within 20 min.
256	360	0.56 c.c. toxin + 1/750 c.c. antitoxic horse serum	78		"	"	Death within 32 min.
85	290	0.56 c.c. toxin + 1/900 c.c. antitoxic horse serum	50		"	"	Death within 25 min.

utes. These symptoms subsided in about thirty minutes. In the third animal questionable symptoms were noted. Other sensitized guinea pigs that had been protected from the second injection of serum with chloral were also reinjected; about 75 per cent. showed slight but definite symptoms.

CHLORAL HYDRATE INJECTED INTRAMUSCULARLY, FOLLOWED BY
NORMAL HORSE SERUM INTRACARDIACALLY.

Sensitized guinea pigs were given chloral hydrate in the same manner mentioned above. Those animals which showed favorable hypnosis were injected directly into the heart with $\frac{1}{4}$ cubic centimeter of normal horse serum. After about one-half minute all the animals showed typical symptoms of anaphylaxis, dying within three to four minutes. All the controls also died within three to four minutes. This experiment was repeated several times with the same results, none of the guinea pigs were protected.

All these animals were autopsied. We found no deaths due to direct hemorrhage. Gross lesions were found in other organs.

TABLE III
INTRAMUSCULAR INJECTIONS OF CHLORAL HYDRATE—FOLLOWED BY NORMAL HORSE SERUM
INTRACARDIACALLY

G. P. No.	Weight	Previous Treatment	Interval in Days	Chloral Hydrate in mg. Intra- muscularly	Second Injection Horse Serum Intra- cardiacallly	Symptoms	Result	Autopsy
353	290	0.56 c.c. toxin + 1/300 c.c. antitoxic horse serum	21	125	$\frac{1}{4}$ c.c.	Typical	Death within 3 min.	No Haemor- rhage in Pericardium
535	330	0.56 c.c. toxin + 1/400 c.c. antitoxic horse serum	51	160	"	"	Death within 4 min.	"
107	300	"	20	140	"	"	"	"
237	280	"	20	120	"	"	"	"
218	520	0.56 c.c. toxin + 1/100 c.c. antitoxic horse serum	29		"	"	Death within 3 min.	"
272	300	0.56 c.c. toxin + 1/400 c.c. antitoxic horse serum	20		"	"	"	"
100	275	"	20		"	"	Death within 4 min.	"

In seeking an explanation for these results we came to the conclusion that a certain optimum content of the drug in the circulation was essential to protect the animal; that possibly the lipoids played an important role in anaphylaxis; that if we injected a sufficient amount of

chloral hydrate directly into the circulation we would obtain a loose physico-chemical combination with the vitally important lipoids of the cells and in so doing change their normal relationship to the other cell constituents, through which an inhibition of the entire cell chemism would result. With this in mind, we injected sensitized guinea pigs weighing between 275 to 300 grams with 30 milligrams of the drug directly into the heart. Even before completion of the injection the animals were under the influence of the drug. After two to four minutes the dose was repeated again into the heart. After again allowing two to four minutes to elapse $\frac{1}{4}$ cubic centimeter of horse serum was injected into the heart.

Chloral hydrate given in this manner protects about 75 per cent. of the sensitized guinea pigs from the second injection when made directly into the heart.

CHLORAL HYDRATE INJECTED INTRACARDIACALLY, FOLLOWED BY NORMAL HORSE SERUM.

Solutions of chloral hydrate were given intracardiacally in divided doses to sensitized guinea pigs. When complete hypnosis was produced $\frac{1}{4}$ cubic centimeter of normal horse serum was given in the same manner.

The dilutions for injection were such that not more than $1\frac{1}{2}$ cubic centimeters were given at one time. These injections were given very slowly, so that the danger from possible dilatation of the heart could be largely ruled out. Also, since intracardiac injections very frequently result in injury to the heart muscle, causing hemorrhages, and since the whole dose does not always enter the heart, experiments were repeated many times. Autopsies were performed in all cases in which death occurred in order to exclude mechanical injury with resulting hemorrhage as the cause of death.

In our preliminary work the selected dose of the drug, which was about one-half of the amount for an intramuscular dose, was given intracardiacally at one injection. This, however, frequently caused the death of the animal. We, therefore, resorted to divided doses at

short intervals. With this method few deaths resulted from chloral injections.

In table No. IV, guinea pig No. 139 received 45 milligrams chloral hydrate intracardiacally; even before the injection was completed the animal was under the influence of the drug. The respiration was deep and irregular for about ten seconds, then short and rapid for about one minute. Three minutes after the first injection 40 milligrams more of the drug were given; the same irregular respirations were observed. Two minutes after the second injection of the drug $\frac{1}{4}$ cubic centimeter normal horse serum was given. A few twitchings at the nose were noticed. Thirty minutes later the animal showed signs of recovery from the effects of the drug and $2\frac{1}{2}$ hours later complete recovery was effected. Guinea pig No. 995 received 40 milligrams chloral hydrate. The animal was under the influence of the drug at the completion of the injection. The respiration was deep and irregular for about ten seconds, then short and rapid. Two minutes after the first injection 40 milligrams more of the drug were given. Two minutes later $\frac{1}{4}$ cubic centimeter normal horse serum was given. No symptoms were noticeable. About $2\frac{1}{2}$ hours later the animal had recovered from the effects of the drug.

Guinea pig No. 16 received 35 milligrams chloral hydrate. The animal was under the influence of the drug at the completion of the injection. Four minutes later 30 milligrams more of the drug were given. Three minutes later $\frac{1}{4}$ cubic centimeter of normal horse serum was given. No symptoms were noticeable. About two hours later the animal had recovered from the effects of the drug.

Guinea pig No. 8 struggled considerably while receiving the 30 milligrams of the chloral hydrate, so that most of the drug did not enter the heart. The animal could run around immediately after the injection. Four minutes later the animal was still walking around. We then gave it 35 milligrams more of the drug in the heart this time. The animal was then under the influence of the drug. Four minutes later $\frac{1}{4}$ cubic centimeter normal horse serum was injected. About one-half minute later typical symptoms developed, the animal dying within four minutes. Autopsy showed no direct hemorrhage. It

appears in this case as though an insufficient amount of chloral had entered the circulation.

Guinea pig No. 24 struggled considerably while receiving the 30 milligrams of chloral. Most of the drug did not enter the heart. The animal could run around. Having in mind the result of the inoculations of guinea pig No. 8, we decided to wait twenty minutes before injecting again. At the end of twenty minutes the animal was in a partial stupor. Thirty milligrams of chloral were given, the animal being under the influence of the drug at the completion of the injection. Four minutes later an additional 30 milligrams of the drug were given.

This was followed three minutes later with $\frac{1}{4}$ cubic centimeter normal horse serum. No symptoms were noticeable. About three hours later the animal recovered from the effects of the drug.

Twenty-four hours later guinea pigs Nos. 139, 995, 16 and 24 received an additional $\frac{1}{2}$ cubic centimeter normal horse serum intracardiacally. All showed typical symptoms after about $\frac{1}{2}$ minute. Respiration ceased within three minutes. The heart continued to beat after respiration had ceased. Autopsies showed none of the deaths due to direct hemorrhage. These experiments were repeated many times with similar results.

TABLE IV
INTRACARDIAC INJECTIONS OF CHLORAL HYDRATE—FOLLOWED BY $\frac{1}{2}$ C.C. NORMAL HORSE SERUM. TIME INTERVAL BETWEEN INJECTIONS 2 TO 4 MINUTES

G. P. No.	Weight	Previous Treatment	Interval in Days	Chloral Hydrate in mg. Intracardially	Second Injection Horse Serum Intracardially	Symptoms	Result	Autopsy
139	380	0.73 c.c. toxin + 1/500 c.c. antitoxic horse serum	50	1st inj. 45 mg. 2d inj. 40 mg.	$\frac{1}{2}$ c.c.	A few scratches at nose	Recovered	
995	340	0.73 c.c. toxin + 1/1200 c.c. antitoxic horse serum	30	1st inj. 40 mg. 2d inj. 40 mg.	"	None	"	
16	310	0.73 c.c. toxin + 1/100 c.c. antitoxic horse serum	50	1st inj. 35 mg.	"	"	"	
8	335	0.73 c.c. toxin + 1/600 c.c. antitoxic horse serum	50	1st inj. 30 mg. missed heart	"	Typical	Death within 4 min.	No hemorrhage in pericardium
24	305	0.73 c.c. toxin + 1/450 c.c. antitoxic horse serum	30	2d inj. 35 mg. 1st inj. 30 mg. missed heart	"	None	Recovered	
13	300	0.73 c.c. toxin + 1/500 c.c. antitoxic horse serum	50	2d inj. 30 mg. 3d inj. 30 mg.	"	Typical	Death within 3 min.	No hemorrhage in pericardium
42	320	0.73 c.c. toxin + 1/1250 c.c. antitoxic horse serum	30		"	"	"	"
33	360	0.73 c.c. toxin + 1/400 c.c. antitoxic	30		"	"	"	"

The fact that those sensitized guinea pigs which, while under the influence of the drug, were protected from the second injection of serum are still sensitized, leads us to the following conclusions: Certain parts of the serum necessary to cause anaphylaxis when injected into the circulation of a sensitized pig, must be rapidly destroyed or eliminated from the circulation. Or, these parts may combine with cells not vitally important to the immediate life of the animal. If this be the case the ferment or zymogen in those protected, sensitized guinea pigs has not all been utilized in destroying or splitting the serum.

In other words, the animal is not in most cases vaccinated, but will react with typical symptoms, followed by death, if, after recovery from the effects of the drug, an additional $\frac{1}{2}$ c.c. of horse serum is given intracardiacally.

We wish to emphasize the fact that from our experiments a certain optimum content of the drug in the circulation is essential to protect the animal. The drug is rapidly destroyed or eliminated from the circulation. This we believe can be shown by allowing twelve to fifteen minutes to elapse between the intracardiac injection of the drug and twelve to fifteen minutes to elapse before injecting the serum. Chloral hydrate given in this manner, with this time allowance will not protect sensitized guinea pigs from the second injection intracardiacally.

TABLE V

INTERCARDIAC INJECTIONS OF CHLORAL HYDRATE—FOLLOWED BY $\frac{1}{2}$ c.c. NORMAL HORSE SERUM. TIME INTERVAL BETWEEN INJECTIONS 12 TO 15 MINUTES

G. P. No.	Weight	Previous Treatment	Interval in Days	Chloral Hydrate Intracardiacally	Second Inj. Horse Serum Intracardiacally	Symptoms	Result	Autopsy
168	300	0.73 c.c. toxin — 1/1400 c.c. anti-toxic horse serum	18	1st inj. 30 mg. 2d inj. 30 mg. 3d inj. 30 mg.	$\frac{1}{2}$ c.c.	Typical	Death within 5 min.	No Haemorrhage in Pericardium
11	290	0.73 c.c. toxin — 1/1700 c.c. anti-toxic horse serum	18	1st inj. 30 mg. 2d inj. 30 mg. 3d inj. 20 mg.	$\frac{1}{2}$ c.c.	"	"	"

CHLORAL HYDRATE INJECTED INTRACARDIACALLY, FOLLOWED BY $\frac{1}{4}$ C.C.
NORMAL HORSE SERUM INTRACEREBRALLY.

Sensitized guinea pigs were trephined and then given divided doses of chloral hydrate in the manner mentioned above. The time allowance between injection of the drug was from two to four minutes. One-quarter cubic centimeter of normal horse serum was then given intracerebrally after the method of Besredka. About 75 per cent. of the sensitized guinea pigs tested in this manner were protected from the second injection intracerebrally.

TABLE VI

INTERCARDIAC INJECTIONS OF CHLORAL HYDRATE—FOLLOWED BY $\frac{1}{4}$ C.C. NORMAL
HORSE SERUM INTRACEREBRALLY. TIME INTERVAL BETWEEN INJECTIONS
2 TO 4 MINUTES

G. P. No.	Wgt.	Previous Treatment	Inter- val in Days	Chlor- al Hy- drate Intra- cardiac- ally	Second Injection Horse Serum Intracerebrally	Symptoms	Result
43	400	0.73 c.c. toxin + 1/750 c.c. antitoxic horse serum	22	1st inj. 45 mg 2d inj. 45 mg.	$\frac{1}{4}$ c.c.	None	Recovered
2	395	0.73 c.c. toxin + 1/500 c.c. antitoxic horse serum	32	1st inj. 40 mg. 2d inj. 40 mg.	"	"	"
26	295	0.73 c.c. toxin + 1/400 c.c. antitoxic horse serum	37	1st inj. 35 mg. 2d inj. 35 mg.	"	"	"
7	415	0.73 c.c. toxin + 1/500 c.c. antitoxic horse serum	32	1st inj. 45 mg. 2d inj. 45 mg.	"	"	"
29	275	0.73 c.c. toxin + 1/300 c.c. antitoxic horse serum	40		"	Typical	Death within 5 min.
333	340	"	32		"	"	"
934	380	"	38		"	"	"

With the different methods of administering the second injection of horse serum into sensitized guinea pigs, which were favorably under

the influence of chloral hydrate, we were able to protect about 75 per cent. In our preliminary communication we stated that we believed that with improved technique in the dosage of chloral hydrate it would be possible to protect 90 per cent. of all fully sensitized guinea pigs.

Now, however, we have come to the conclusion that, if the smallest dose of serum which will just kill any animal of a given series of sensitized guinea pigs regularly, be injected into the remainder of the animals of the same series of sensitized guinea pigs, when properly under the influence of chloral hydrate, that protection will be afforded in practically all cases.

AN OCCASIONAL COMPLICATION OF THE PASTEUR ANTIRABIC TREATMENT

By D. W. POOR, M.D.

Since the Pasteur antirabic treatment has come into general use cases of obscure illness have been from time to time reported during the course of the treatment, which in a few rare instances have ended in death. Some of these were undoubtedly intercurrent conditions not connected with the antirabic treatment. Others may have been due to bacterial contamination, but in still others there is sufficient similarity in the symptoms to awaken the suspicion that they may have been due to the treatment itself. The older writers considered such illness to be due to the original canine infection modified by the Pasteur injections, or even to a form of rabies produced by the injections themselves. The manifold symptoms due to neurasthenia and hysteria occasionally appearing in those taking the Pasteur treatment have still further complicated the subject so that it is not altogether surprising that one reads that the "Pasteur treatment may produce blood poisoning, neuritis, ascending paralysis, insanity and even rabies itself."

It was not until 1905 that an extensive investigation was made of this subject. In that year Remlinger published an article in the *Annales de l'Institut Pasteur* on paralytic accidents occurring during the course of antirabic treatment. The article was the result of an investigation among the various European laboratories and institutes, following a case which occurred in his own practice. The record of Remlinger's case is as follows:

A boy 13 years old, bitten on the right thigh by a dog diagnosed rabid by a veterinary surgeon. Treatment was begun a week later. On the morning of the twelfth day of treatment he complained of severe pain in the muscles and joints, and a sensation of great weakness in the legs. He attributed the symptoms to a cold shower bath taken the night before. The injections were continued. The next day the pain was no better, there was complete paralysis of the legs, with retention of urine, necessitating catheterization. The antirabic treatment was suspended.

There was no fever. The following day his condition was worse. The pain extended to the neck and face, and paralysis began in the arms. The fourth day the paralysis of the arms was more pronounced. During the next week the pain diminished, and then disappeared. The tactile sensibility was preserved, but reflexes abolished. Retention of urine and constipation persisted. The paralysis of legs was still complete, that of the arms incomplete. On the thirteenth day movement slowly returned to the arms, but the paralysis of the legs remained. Retention of urine was replaced by incontinence. A week later the patient could move the great toe of the left foot. The following day he could move all the toes of the left foot. Later he moved the left leg and then was able to move the great toe of the right foot. In another week the patient was able to raise himself and take a few steps. From this time convalescence was rapid and he left the hospital thirty-eight days after the onset of his illness. There remained a little anemia and weakness, but no muscular atrophy.

Remlinger has collected the records of about 40 cases which showed distinct objective symptoms. These occurred among a total of approximately 100,000 persons treated. Two were fatal, one after symptoms lasting one month and one after nine months. Analysis of the parts of the body involved is shown in the following table:

Legs and sphincters.....	17	cases
Legs alone	6	"
Legs, sphincters, arms, face.....	4	"
Legs, sphincters, face	4	"
Arms and face.....	1	case
Arms alone	1	"
Face alone	1	"
Legs, arms, sphincters and bulbar symptoms.....	1	"
Legs and face.....	1	"
Legs and sphincters and bulbar symptoms.....	1	"

A résumé of the symptoms as given by Remlinger is as follows:

The onset may be from the eighth day of treatment to the end of the first week following it. There is usually more or less paralysis of the legs with or without involvement of the bladder and rectum. These symptoms are frequently preceded by lancinating pains in the limbs and occipital headaches. The sensory symptoms are variable. There may be hypersensibility of the skin with increase of reflexes or anesthesia

with loss of reflexes. Various forms of paresthesia are apt to occur. The symptoms may be confined to the legs or assume the type of an ascending paralysis. The muscles of the face may be involved and bulbar paralysis with symptoms of dyspnoea, tachycardia and dysphagea may occur. Even in these last cases recovery is possible. In the majority of cases, after about three weeks the symptoms ameliorate and recovery is complete in several days or weeks. One case lasted six months and one is reported to have been unimproved at the end of six years. Apparently the continuation of the treatment has no effect upon the course of the condition.

In 1908 Remlinger¹ reports two more cases, Masson² one case, Pam-poukis³ ⁴ three cases, and Babes⁵ and Mironescue report a fatal case. The history of the latter is as follows:

A woman 40 years of age, thin and of nervous temperament, was severely bitten by a rabid dog and began the treatment six days later. After 14 days of treatment of medium intensity, the course was interrupted by the appearance of paralysis of the legs without hydrophobia. The paralysis took on the character of the ascending type of Landry. The patient was sent to the hospital, where she died the next day. The autopsy showed lesions affecting mainly the lumbar cord. There were areas of softening and hemorrhage, involving both the gray and white matter.

REPORT ON ADDITIONAL CASES

Out of a total of 2,300 patients treated I have encountered twelve cases presenting this interesting complication. I have included those which gave symptoms suggesting a mild neuritis as well as those which seemed to indicate an involvement of the cord, as the process appears to be identical in all, and merely variable in degree.

Unfortunately it has been impossible to learn the subsequent history of most of these cases.

(1) A. E., male, 78 years of age, received two small wounds of the wrist from a dog in which the diagnosis was uncertain. Treatment was sent* for 21 days. During the last two or three days of treatment and

*Virus prepared at the laboratory and sent by mail to the attending physician, who carries out the treatment.

for the next two weeks patient had numbness and weakness in the upper and lower extremities.

(2) S. McN., male, aged 33, severely bitten on the hand by a rabid dog. Treatment sent for 22 days.* On the seventeenth day of treatment there were pains and other symptoms of neuritis in the left leg. This was followed by retention of urine for several days, necessitating catheterization. Patient confined to bed. At the end of 15 days the symptoms had about disappeared.

(3) H. A., male, 25 years old. Scratched by a cat in which the diagnosis was uncertain. Treatment sent* for 18 days. During the last six days there was severe pain in the sacral region. Patient failed to report further.

(4) J. B. M., female, 38 years old. Scratched on the hand, which it was thought might have been infected by the saliva of a mad dog. Treatment sent* from September 8 to September 28. On October 9 the attending doctor reports that for the past few days patient has had pain and numbness in the extremities. No further history obtainable.

(5) W. P., male, 42 years old. Four or five superficial wounds on hands inflicted by dog with clinical symptoms of rabies. Treated 21 days; the last part of the treatment sent;* the doctor reports patient has severe neuralgia, especially in the legs, for the two weeks following treatment. Duration unknown, as further information was not received.

(6) Mrs. B., 42 years old. A slight wound came in contact with the saliva of a rabid dog. Patient treated 15 days. Paresthesia, general depression and pains throughout body accompanied by marked general depression. These symptoms were said to have lasted a year, during part of which time patient was under treatment at a sanitarium. The history of this case is somewhat complicated by the onset of the menopause during this period.

(7) Mr. S., aged 52, and daughter, aged 17. Wounds on fingers, fairly severe. Diagnosis of dog doubtful. Treatment sent* for 14 days and then stopped on account of the following symptoms, especially pronounced in Mr. S.: There were severe headache, pains in legs and

*Virus prepared at the laboratory and sent by mail to the attending physician, who carries out the treatment.

arms, and profound general weakness. About 20 months later the attending physician writes as follows: "Mr. S. states that five weeks after receiving the last injection he had numbness and swelling of the right hand, the numbness especially along the distribution of ulnar nerve. Symptoms disappeared in about two months. A numb sensation along the outer aspect of the left thumb has persisted until the present time. He complains of pain between the shoulders extending up to the right occipital region. Patient further states that for about two months after taking treatment he had partial paralysis of right wrist, extension being difficult. Even at the present time he is frequently troubled with sciatic pains. All of these symptoms appear to be abating during the past few months.

"Miss S. had no numbness, but the pain in the dorsal region, the occipital and sciatic pains were similar to her father's. During the past three months, however, she has been free from pain. At the time of treatment she weighed 90 pounds. At the present time she weighs 115 pounds, and appears to be in perfect health. I think Mr. S. is somewhat of a neurasthenic."

(8) J. R., male, 50 years old. Two scratches on the hands which it was thought might have been infected with the saliva of a rabid dog. Treatment sent* for 17 days. On fifteenth day of treatment patient complained of numbness in both thighs and legs, mainly the left, which lasted three weeks.

(9) Name unknown. Coachman. Wounds exposed to the saliva of a rabid dog. Treatment sent* for 18 days. During the latter part of the course patient complained of gastric indigestion, and numbness in the extremities. Duration and further history unknown.

(10) R. T., 38, male. Very slightly bitten on the hand by a dog in which the diagnosis was doubtful. Treated at the laboratory 15 days when patient complained of severe pain in back and down both thighs. Treatment stopped. On examination three days later patient was found to be suffering from severe lumbar and sciatic pain which at night prevented sleep. There was slight difficulty in walking, which was ap-

*Virus prepared at the laboratory and sent by mail to the attending physician, who carries out the treatment.

parently due to the pain. No distinct signs of paralysis noted. Skin sensations and knee jerks normal. No bladder and rectum involvement. Pronounced occipital headache. Later the pains and paresthesia extended to arms and face. Insomnia continued troublesome. Patient exhibited a variety of neurasthenic symptoms and finally went to a sanitarium. Recovery after several months.

(11) C. W., aged 4. Badly bitten on face by rabid dog. Treatment sent 11 days later. There is some question as to the nature of this case, but it is classified with the others for want of a better understanding of it. After 17 days of treatment child was sent to the hospital suffering with severe headache and marked restlessness. Temperature 99.6, pulse 96. The following day there were dysphagia, difficulty in articulation, marked twitching of muscles of face, severe pain in the back of the neck and marked constipation. Patient seemed very rigid. Third day the patient was quieter, but apparently unable to speak. Temperature 101.8. Urinalysis negative. Fourth day a small abdominal abscess was opened. Headache continued. Urination involuntary. Later in the day the twitching disappeared, and patient relaxed. Fifth day twitching returned, urine still passed involuntarily. Sixth day patient was more comfortable, but there was occasional twitching. Seventh day, temperature 99.4. Twitching had stopped. Nine days later patient discharged, well.

(12) H. K., male, aged 63. Bitten quite severely on the arm by a dog which, though sick, was shown later not to have been rabid. Patient was a large, well preserved man. Previous history unimportant. Treatment began five days after the bite and continued for 12 days at the laboratory. Although patient's wife stated later that he had complained of certain symptoms almost from the beginning, he made no complaint at the laboratory, and on the day of the last injection he seemed normal. The following report was sent by the family physician:

"After the second injection patient began to complain of general weakness, pain at the points of injection and restlessness at night. His complexion seemed sallow and he tired easily after slight exertion. On the day after the twelfth injection he walked, with the aid of a cane, to my office, complaining of digestive disturbance, particularly flatulence.

He also complained of numbness in the legs. That same night I was summoned to the patient's house and found him sitting upright in a chair with anxious countenance, marked restlessness and loss of strength. The pains and paresthesia were about the same as in the morning. He was then able to swallow liquids, but solids, such as bread, caused paroxysms of cough. There was no fear of water. A most pronounced change since morning was the development of a profound prostration. In spite of veronal and trional he passed a very restless night, appearing the next morning very weak. He was unable to sit up in bed and could speak only in a whisper. Examination of the throat was negative. Tongue was protruded without difficulty. Temperature by mouth was 96, pulse about 70, and of good quality. At 10 A. M. patient was given a small sip of water which produced violent cough, dyspnoea and cyanosis. Pulse during the attack 66, and of good quality. At 2 P. M. his condition was still more grave and a consulting physician was called. During the examination there developed extreme dyspnoea, a weak, ineffectual cough, great restlessness and air hunger. Patient constantly threw himself about. Expression extremely anxious. About 10 minutes later respiratory paralysis set in, the heart continuing to beat about two minutes longer. Its action at first was quite strong and regular, gradually becoming slow (about 18 per minute) and weaker. Death occurred at 3.10 P. M.

Practically no changes were found in the brain and cervical cord of this case either in the gross inspection or microscopic examination of sections. No examination was made of the lower portion of the cord. The body was injected with an embalming fluid containing formalin, before autopsy. A portion of the brain apparently unaffected by the fluid was emulsified and injected into guinea pigs without result.

CAUSE OF THE SYMPTOMS.

The various hypotheses which have been advanced to account for these symptoms are:

(1) Bacterial contamination, (2) hysteria, (3) modified canine rabies, (4) rabies due to the treatment, (5) rabic toxin.

Bacterial contamination may be dismissed as a cause for the reasons that cultures of the emulsions do not show the presence of bacteria,

moreover, if this was the cause, more individuals would probably be affected at the same time, and further, there would not be the almost uniform tendency to recovery.

Hysteria is out of the question. In one of our cases, the course of the illness was somewhat modified by symptoms of hysteria, but these set in after the characteristic neuritis. In none of the other recorded cases were symptoms of hysteria present. Furthermore, if due to hysteria the symptoms would not be of so uniform a character. Neither alcohol nor syphilis can be held accountable. All of our cases were in excellent physical condition.

Modified canine rabies may be excluded since in quite a number of instances the animal inflicting the bite was shown not to be rabid.

Rabies due to the treatment is equally unsatisfactory as an explanation, since the symptoms often set in even before they would after subdural infection with fixed virus. Further, the brain substance of those cases going to autopsy has been incapable of producing rabies. Babes has been able to produce similar paralysis in animals by the injection of heated virus in which the rabic organism is dead. He has also noticed these symptoms in a man who had received only virus which was heated to 75° or 80° and cords dried no shorter time than eight days.

I have observed the death of guinea pigs injected with virus killed by heat as well as that killed by chloroform. The paralyzes noted by Babes were not observed. Nothing of importance was seen at autopsy. The brain substance was incapable of producing rabies. An interesting fact is that, as in humans, only one or two out of a group of seven or eight pigs injected would be affected.

The most plausible theory thus far advanced is that the symptoms are due to the presence of a toxin in the emulsion, this toxin being characteristic of the rabic virus. The presence of this toxin together with an idiosyncrasy on the part of the patient is held accountable for the symptoms by Babes and Remlinger.

Pampoukis, while accepting the toxin part of the theory, offers as a substitute for "idiosyncrasy" the following idea: The rabies toxin is taken up by the phagocytes and so changed by them in a biochemical way that it can no longer act on the nervous system. When, however,

the organism is so weakened that the phagocytes cannot perform their function, the toxin reaches the nervous system unchanged and so irritates it as to produce the symptoms. According to him the most important cause of this "upsetting of equilibrium" in the body is chilling of the patient, especially by cold bathing. In six of the 40 cases recorded by Remlinger cold bathing immediately preceded the onset of symptoms. In the two cases reported by Pampoukis paralysis followed a cold bath in one, and chilling of the body in the other.

No attempt has been made to seek for this factor in the etiology of our cases, although it is recalled that one of those having most pronounced symptoms was accustomed to swimming long distances in the sea during the period of treatment.

Mariensco supposes that the cause may be a cytotoxin due to the presence of a foreign protein and intimates that the symptoms would follow the injection of normal nerve substance. I have been unable to find any animal experiments in literature which directly prove this.

On the other hand, Babes has injected not only animals but persons with large amounts of normal nervous substance without producing any paralysis. In a series of sixteen guinea pigs which I injected intraperitoneally with large amounts of emulsion of dog brain for the production of neurolytic serum, I saw no bad effects whatever. Babes does not believe that the amount and strength of the virus are factors because he has not noted the symptoms in those taking the strongest treatment. A more convincing argument would have been produced if a detailed analysis of his cases had been given, especially with reference to the proportion of severe and mild cases treated.

It seems to be generally admitted that these paralytic accidents do not accompany the dilution method of Hogenes, in which very small amounts of nervous matter are injected.

I feel that while idiosyncrasy is a most important factor, the character of the virus is also of importance.

One of our cases occurred in 1906, four in 1907 and seven occurred during the nine months between February and October, 1908. During the succeeding nine months there have been no cases. It is a coincidence that may have some bearing, that about October, 1908, we began to inoculate rabbits from the cord rather than from the brain of the rabbit

of the preceding series. In this way we undoubtedly used a weaker virus, as the rabbits did not show the initial symptoms until a day and sometimes two days later than by the other method. During this time also we have been careful to give smaller and weaker doses to those who were but slightly bitten.

It is a curious fact that out of our 12 cases nine were either but very slightly bitten, or only had wounds exposed to the saliva of rabid dogs, or else were bitten by animals proved not to be rabid. Only two were severely bitten by rabid animals, and in one the diagnosis was unknown.

In conclusion it may be said:

(1) that in a small proportion of patients undergoing Pasteur antirabic treatment, a complicating affection of the nervous system occurs, the symptoms of which vary from what is apparently a mild neuritis to an acute ascending paralysis. While the prognosis is almost always good, a certain small undeterminable proportion of the cases terminates fatally.

(2) That these symptoms are probably due to substances in the nature of toxins associated with the rabic virus.

(3) That in the absence of a clear understanding of these cases the most important predisposing condition must be regarded as personal idiosyncrasy.

(4) That exposure to cold and especially cold bathing appears to be an associated causative factor.

(5) That the variations in the virus (strength, etc.) probably are of importance in the etiology.

(6) That while these complications should not be considered in the case of those who evidently need the Pasteur antirabic treatment, account should be taken of them in deciding on the advisability of this treatment for those in whom infection is doubtful.

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THE IMMUNIZING PROPERTIES OF KILLED RABIES VIRUS

D. W. POOR, M.D.

The action of dead rabies virus in producing immunity is of interest not only from a practical but from a theoretical standpoint. A consideration of the best methods for combating infection in man as well as the practicability of vaccinating dogs against rabies depends to a considerable extent on the availability of dead virus for this purpose.

The bearing of this question on the theoretical considerations regarding the nature of rabies infection is ably discussed by W. F. Harvey and Anderson McKendrick in their work on the theory and practice of antirabic immunization.¹ These authors conclude that the immunizing property of dried cords is directly proportional to their infectivity and inversely proportional to their loss of water, and that, therefore, cords dried nine days and more are practically useless for immunizing purposes.

On the other hand, Fermi² as the result of his experiments recommends the use of dead virus only, his plan being to kill the virus with carbolic acid and store the emulsions for future use.

Otto Heller in his work on protective inoculation against rabies, Jena, 1906, gives the experience of others in the use of virus killed in various ways, e. g., heat and by glycerin, and his own experiments in the use of virus killed by grinding according to the method of McFadyen. The brains of rabbits dead with fixed virus infection were hardened by freezing with liquid air. They were then ground up in a metallic mortar operated by a motor, for several hours. The mortar with the brain material was kept cold during the operation by exposure to liquid air. He found that the virus was killed by three hours grinding but not by one and one-half hours grinding.

He treated ten rabbits by five or six subcutaneous injections of this virus, the injections being given several days apart. Six of the animals survived, one died of rabies and three from other causes. All the controls died of rabies.

It is a rather curious point that in the treatment of the rabbits which varied slightly in detail in each case, at least one injection of material ground for only two hours was given in each instance. According to his previous experiments it is uncertain whether or not this was killed.

Heller's conclusions are (1) The live virus is not absolutely essential for the production of immunity. (2) McFadyen's method is the best, as it destroys the organisms without changing their action. (3) It is possible to produce paralysis and marasmus (toxic symptoms) by means of killed virus.

The following tests were made, to determine to what extent immunity could be produced by virus killed in various ways and to gain some idea of the length of time each virus would preserve its immunizing properties.

Three kinds of virus were used. First, an emulsion in salt solution prepared from the brain of a fixed virus rabbit. This emulsion was killed by heating to 60° C. for twenty minutes. The immunizing strength of this emulsion was tested in the fresh state and after having been kept in the ice-box at 45° F. for a week. Second, emulsions made from the dried cords of the ordinary Pasteur series. Cords dried twelve, eleven, ten and nine, eight and seven days were used. In another experiment similar cords with the exception of the eight and seven day cords were used. Third, the brains of fixed virus rabbits were spread in thin films and the virus dried and at the same time killed by exposing it to dry heat at 50 to 53 and 60 to 63 as shown in the following table. This virus was stored in the ice-box before being used in one case for eleven days, in the second test for two and one-half months. Emulsions were then made in salt solution as in the ordinary Pasteur method.

In these tests the guinea pigs were first treated for varying lengths of time, ten to twenty days. At the completion of the series of injections (two c.c. each day) a period of rest from six to sixteen days was allowed for the development of possible immunity. At the end of this time, each guinea pig of the series, together with an equal number of controls, was given from one to one and a half cubic centimeters

of a thick emulsion of street virus in the region of the right sciatic nerve.

The following table shows the results of nine tests upon guinea pigs treated with these three forms of killed virus. (See page 134.)

It appears, as would be expected, that in a general way the mortality in the treated animals varies according as they are treated for a longer or shorter time, according to the severity of the infection by the street virus as shown by the mortality and incubation of the control pigs, and according to the length of time allowed for the development of immunity.

It may be said that the results shown in the table indicate.

(1) What antirabic immunity may be produced by these three varieties of killed virus.

(2) That the "Pasteur virus" gives a better immunity than the brain virus emulsions heated to 60°.

(3) That this latter virus loses much of its immunizing power by being kept at 45° F. for a week.

(4) That by drying the fixed virus brain and at the same time killing it by heat, a virus is obtained which, when kept in the dry state at 45° F. for two months, has still a very considerable immunizing power.

It may be added that the minimum degree of heat required to kill the brain virus, as used in the method described above, has not yet been determined. Exposure to from 50 to 53° C. for eighteen hours kills and dries the virus. Exposure to 36° C. for twenty-four hours dries but does not kill it. Emulsions made from this latter virus kill guinea pigs in 6-7 days when inoculated subdurally.

We are at present preparing the killed and dried virus from the brains of our fixed virus rabbits, and this will be tested on a series of dogs with both before and after infection.

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² Comptes Rendus, 1908.

³ Otto Heller—Die Schutzimpfung gegen Lyssa (Fischer). See also Annales de L'Institut Pasteur, 1908.

HEATED VIRUS EMULSION STORED FOR ONE WEEK AT 45 DEG. F. BEFORE USE

4	7	13 days	1½ c.c. 6 days after end of treatment	57% 14 days	85% 16 days
5	6	13 days	1 c.c. 11 days after end of treatment	83% 17 3/5 days	100% 15 2/3 days

PASTEUR VIRUS

Test Number	No of Pigs Treated	Length of Treatment	Virus Used	Amount of Infecting Virus and Time at Which It Was Given	Mortality	Mortality and Average Incubation of Controls
6	7	15 days	Cords dried 12-7 days	1 c.c. 15 days after end of treatment	0	100% 18 5/7 days
7	6	12 days	Cords dried 12-9 days	1 c.c. 11 days after end of treatment	0	100% 15 2/3 days

BRAIN VIRUS, HEATED, DRIED AND STORED

8	5	12 days	Brain virus heated for 18 hrs. between 50 and 53 deg. C. and stored 11 days	1½ c.c. 11 days after end of treatment	0	100% 13 days
9	7	12 days	Brain virus heated 19 hours between 60 and 63 deg. C. and stored 2½ months	1½ c.c. 12 days after end of treatment	28% 14½ days	100% 13 2/7 days

REPORT OF CASES RECEIVING PASTEUR ANTIRABIC
TREATMENT DURING 1908

D. W. POOR, M.D.

Treated at Laboratory.

	Diagnosis of Rabies in the Animal Certain	Diagnosis Probable.	Diagnosis Uncertain.	Animal Not Rabid,
Bitten on head.....	9		1	3
Bitten elsewhere.....	92	6	32	12
Contact of wounds with saliva of animals.....	11	1		3

Treatment Sent in City.

Bitten on head.....	1			
Bitten elsewhere.....	14	6	5	1
Contact cases.....	20	2		

Treatment Sent out of City.

Bitten on head.....	45	6	6	2
Bitten elsewhere.....	327	32	57	2
Contact cases.....	50			
	<hr/>	<hr/>	<hr/>	<hr/>
	569	53	101	23
Total, 746				

CASES TREATED, BY MONTHS

January	45	August	62
February	38	September	65
March	80	October	67
April	61	November	47
May	67	December	54
June	82		<hr/>
July	78		746

Residents, 200.
Non-residents, 546.

In addition a considerable number of persons took the treatment for a short time, pending the diagnosis in the dog. These cases are not included in the above report.

RECORD OF CASES WHICH DIED IN SPITE OF TREATMENT

	Age.	Location of Bite, Etc.	Period of Treatment.	Onset of Rabies.
Mrs. D.	42	Three penetrating wounds on bare arm, Aug. 30, A.M. Treated by doctor $\frac{1}{2}$ hour later with carbolic acid, followed by alcohol and at laboratory, 3 days later with HNO ₃ .	Sept. 2 to 22 (both dates inclusive)	Pain in the arm, Sept. 23. Difficulty in swallowing water Oct. 1. Death a few days later.
G.R.	34	Five wounds on fingers, four penetrating and one lacerated. Cauterized with AgNO ₃ Dec. 12.	Dec. 26 to Jan. 16	March 4, unable to drink. Died March 6.
J.W.	20	Two wounds on right side of nose and two on upper lip. March 2, cauterized immediately with carbolic acid.	March 8 to April 3 Patient skipped six days of treatment, four days at one time and two at another.	Died May 3
E.P.	5	Bitten on right ear, incised wound $\frac{1}{4}$ inch deep, Jan. 13. Cauterized one hour later with AgNO ₃ .	Feb. 13 to Mar. 9	First symptoms of rabies, Mar. 9. Died Mar. 12.
Mr. F.	77	One penetrating wound on back of hand of moderate severity, and another fair sized wound on hand. Cauterized two hours later with HNO ₃ .	Apr. 14 to Mar. 3	First symptoms of rabies, July 14. Died July 20.

Three of the patients died of rabies after the period of observation. This gives a mortality of 4-10 of 1 per cent. in the total number treated in which the biting animal was certainly or probably rabid.

THE POWER OF CERTAIN DRUGS TO INHIBIT RABIC INFECTION

D. W. POOR, M.D.

The success obtained in the treatment of certain forms of trypanosome infection and also in that of syphilis by the use of atoxyl and mercury suggested the experimental trial of these drugs in the infection of rabies.

In the *Zeitschrift für Hygiene*, Vol. LIX, 1908, Heymann reports his experiments with atoxyl in attempting to prolong or prevent the onset of rabies. Rabbits were used, and the animals were first inoculated with rabies virus and then for several days treated with atoxyl, some subcutaneously, others intravenously, and still others by mouth. The action of atoxyl on rabies virus was also tested in vitro. His conclusions were that no effect was produced by this agent, either in the animal or in vitro, when given in doses that were non-toxic for the rabbits.

Since it was the combined use of atoxyl and mercury that had been successful in trypanosomiasis, we tried the effects of these agents together. In addition we studied the effect of chinosol and mercuric iodide, antiseptics theoretically serviceable for constitutional use.

In the experiments of Benjamin Moore, reported in the *Biochemical Journal*, 1907, rats were infected with trypanosomes, then given $\frac{1}{2}$ c.c. of a 5 per cent. solution of atoxyl and later from 1.5 to 2.5 c.c. of a 1-1000 solution of mercuric chloride in divided doses.

The drugs were tested in the following strengths: Atoxyl, 5 per cent. solution; chinosol, $\frac{1}{2}$ per cent. solution; mercuric iodide, 1-5000 solution; bichloride of mercury, 1-1000 solution.

Preliminary experiments showed that the non-toxic doses of these drugs for guinea pigs were:

Atoxyl, $\frac{1}{2}$ c.c.

Chinosol, 4 c.c.

Mercuric iodide, 1 c.c.

Bichloride of mercury, $\frac{1}{2}$ c.c.

TEST I

December 5th, 28 guinea pigs were given 1 c.c. of an emulsion of street virus in the leg.

Atoxyl and Mercuric Chloride

(a) On December 7th, seven of the pigs received $\frac{1}{2}$ c.c. each of a 5 per cent. solution of atoxyl, and on December 9th, $\frac{1}{2}$ c.c. of 1/1000 solution of HgCl_2 .

- Pig No. 1, died December 10th, not of rabies.
- Pig No. 2, died January 1st, rabies.
- Pig No. 3, died December 11th, not rabies.
- Pig No. 4, died January 2d, rabies.
- Pig No. 5, remained well.
- Pig No. 6, developed rabies January 16th.
- Pig No. 7, developed rabies December 30th.

Mercuric Chloride

(b) On the same date seven of the pigs received $\frac{1}{2}$ c.c. each of 1/1000 HgCl_2 solution. On December 9th this was repeated.

- Pig No. 1, died of rabies January 28th.
- Pig No. 2, remained well.
- Pig No. 3, remained well.
- Pig No. 4, remained well.
- Pig No. 5, developed rabies December 28th.
- Pig No. 6, developed rabies January 8th.
- Pig No. 7, developed rabies December 31st.

Controls

- Pig No. 1, developed rabies January 4th.
- Pig No. 2, developed rabies December 31st.
- Pig No. 3, developed rabies December 29th.
- Pig No. 4, developed rabies December 23d.
- Pig No. 5, developed rabies December 31st.
- Pig No. 6, died of rabies January 11th.
- Pig No. 7, remained well.

Chinosol

(a) December 28th, 22 pigs were infected in the leg with 1 c.c. of emulsion of street virus. Of this number eight were inoculated subcutaneously with a 1/200 solution of chinosol as follows: December 31st, 1 c.c.; January 2d, 1 c.c.; January 4th, 1 c.c.

- Pig No. 1, remained well.
- Pig No. 2, remained well.
- Pig No. 3, died of rabies January 12th.
- Pig No. 4, developed rabies January 15th.
- Pig No. 5, died of rabies January 27th.
- Pig No. 6, developed rabies January 31st.
- Pig No. 7, remained well.
- Pig No. 8, remained well.

Chinosol, Locally

(b) A second series of seven pigs received the chinosol solution locally in the region of the inoculation as follows: December 31st, 1 c.c.; January 2d, 1 c.c.

- Pig No. 1, remained well.
- Pig No. 2, remained well.
- Pig No. 3, remained well.
- Pig No. 4, remained well.
- Pig No. 5, developed rabies January 20th.
- Pig No. 6, died of rabies January 11th.
- Pig No. 7, died of rabies January 16th.

Controls

Pig No. 1, remained well.
 Pig No. 2, remained well.
 Pig No. 3, remained well.
 Pig No. 4 remained well.
 Pig No. 5, developed rabies January 17th.
 Pig No. 6, died of rabies January 20th.
 Pig No. 7, died of rabies January 18th.

Iodide of Mercury

(c) Fourteen pigs inoculated in leg with 1 c.c. of street virus emulsions on February 6th. On February 7th seven pigs received 1 c.c. of a 1/5000 solution of mercuric iodide. On February 8th, 1 c.c. was given in site of inoculation, and on February 11th another injection of 1 c.c. was given subcutaneously.

Record

Pig No. 1, developed rabies February 2d.
 Pig No. 2, developed rabies February 26th.
 Pig No. 3, developed rabies March 15th.
 Pig No. 4, developed rabies February 29th.
 Pig No. 5, developed rabies February 19th.
 Pig No. 6, developed rabies February 25th.
 Pig No. 7, remained well.

Controls

Pig No. 1, developed rabies February 20th.
 Pig No. 2, developed rabies February 20th.
 Pig No. 3, developed rabies February 18th.
 Pig No. 4, developed rabies March 2d.
 Pig No. 5, remained well.
 Pig No. 6, remained well.
 Pig No. 7, remained well.

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THE DISTRIBUTION OF BACTERIA IN BOTTLED MILK AND ITS APPLICATION TO INFANT FEEDING*

MIDDLE MILK MIXTURES

By ALFRED F. HESS, M.D., NEW YORK

So many experiments have been made and so much has been written upon the bacteriology of milk and the methods of obtaining pure milk, that it did not occur to me to inquire whether the question of the distribution of bacteria in milk was definitely understood. Some months ago, however, in the course of an investigation of tuberculous contamination of milk, I had occasion to centrifuge small amounts of milk and to make smears from the surface of the cream which formed in a firm layer at the top of the tube. Although I had realized that the cream of the milk contains relatively many bacteria, I was impressed with the fact that these smears, on staining, showed an immense number of various organisms, including numerous chains of streptococci. This led me to inquire more closely into the distribution of the bacteria in our bottled milk, where the cream has risen by the natural force of gravity. I set myself the task of finding out where the bacteria in an ordinary bottle of milk may be found; not only whether, and to how great an extent, they are to be found at the top or at the bottom, but whether the divers layers, if we divide the cream arbitrarily in strata, contain relatively constant quantities.

The only method applicable to this end seemed to be the removal of definite amounts from the bottle, for the purpose of making separate bacterial estimations. With this object in view, I obtained numerous bottles of different brands of milk and pipetted off successive layers of cream or skim-milk. The technic of counting was that in use at this laboratory. Although a pipette was employed in most instances to remove the milk, in two series a 1-ounce dipper was used, and in two others simple pouring was employed.

The tables will best explain the results obtained. A glance at the section A of Table I shows at once that the cream contains by far the

* Read before the New York Academy of Medicine, Section on Pediatrics, April 9, 1908.

TABLE I
BACTERIA PER C.C. OF VARIOUS LAYERS OF BOTTLED MILK

A	(1st 50 c.c.)	(2d 50 c.c.)	(3d 50 c.c.)	(4th 50 c.c.)	(5th 50 c.c.)	(Lowest 50 c.c.)	
1	130,000	101,000	48,000	18,000	3,000	7,000	
2	48,000	34,000	27,000	14,000	1,000	4,000	
	(1st 100 c.c.)	(2d 100 c.c.)					
3	38,000	6,000					
	(1st 100 c.c.)	(Remainder)					
4	36,000	5,000					
	(Upper $\frac{1}{2}$ cream)	(Lower $\frac{1}{2}$ cream)	(Remainder)				
5	116,000	17,000	1,000				
B	(1st oz.)	(2d oz.)	(3d oz.)	(4th oz.)	(5th oz.)	(6th oz.)	
1	108,000	70,000	58,000	41,000			
2	105,000	68,000	20,000	8,000	11,000	10,000	Poured
3	124,000	89,000	108,000	58,000	10,000	2,000	Dipped
4	243,000	184,000					
5	1,540,000	1,226,000	160,000				
C	(1st oz.)	(2d 2 oz.)	3d 2 oz.)	(Remainder)			
1	235,000	96,000	76,000	40,000			
2	115,000	36,000	7,000	6,000			Poured
3	102,000	57,000	17,000				Dipped

greatest number of bacteria, and that the skim-milk, even in the lowest portions of the bottle, is relatively free. This has been brought out by Freeman and others. Heretofore, however, the cream has been regarded as a homogeneous unit, containing numerous bacteria in uniform suspension. Closer inspection of the table reveals that this is not the case; that in every instance the bacterial contamination is greatest in the uppermost cream, becoming always less and less as we approach the lean milk. This is equally true even in the milk which has been poured. The first ounce contains more bacteria than the second ounce, the second more than the third, and so on. The first two ounces, however, form a nidus for the greatest number of organisms. Section C of Table I demonstrates this point, but as it is essential to the practical application which I shall later suggest, I have framed a second table (Table II), which comprises counts of the first two ounces, the second

TABLE II

1st 2 oz.	2d 2 oz.	Remainder.
93,500	49,000	
86,500	14,000	
235,000	96,000	40,000
115,000	36,000	6,000
102,000	57,000	
1,383,000		160,000

two ounces, and, in some instances, of the remainder of the bottle. I would like to call attention to the last count of the series, where the first two ounces of the cream contained 1,383,000 bacteria, and the remainder of the bottle only 160,000 per c.c. In this instance, by removing the first two ounces, we freed the milk of about eight millions of bacteria. This principle applies not only to milk of high bacterial count, but also to milk coming well within the range of certified milk, such as number 2 in group C of the first table, in which the average count of the bottle was less than 15,000 per c.c. Here the first two ounces contained 115,000 germs per c.c., so that when we include this portion in our formulæ we, by this means, contaminate the remainder of our milk. Or, if we add the next two ounces, using the upper four ounces, as some advise, we even then make use of cream containing 75,000 bacteria to the c.c.

I was interested to ascertain whether tubercle bacilli were likewise carried upward toward the surface of the cream by the rising of the fat globules. For this purpose I prepared a homogeneous suspension of bovine bacilli, with which I infected "loose" milk. Two to five c.c. to the quart were used, and the milk was allowed to stand 24 hours. On the following day four guinea pigs were inoculated with specimens taken respectively from the first two ounces, the second two ounces, as well as from the skim-milk and lowest ounces in the bottle. The inoculations were made intraperitoneally, and one ounce injected, with the result that many pigs died of infection before tuberculosis could develop. I have prepared a table (Table III) of five such experiments, realizing that they are inconclusive, but believing that they show the preponderance of bacilli in the upper two ounces. In order to obtain

TABLE III
ANIMAL INOCULATION WITH TUBERCULOUS MILK

Experiment.	I.	II.	III.	IV.	V.
1st 2 oz. cream....	*	Tb.	Tb.	Tb.	Tb.
2d 2 oz. cream....	No Tb.	*	No Tb.	Tb.	2*
Skim-milk.....	No Tb.	No Tb.	No Tb.	No Tb.	No Tb.
Lowest 2 oz.....	*	No Tb.	No Tb.	Tb.	No Tb.

* Signifies that pig died of early septic infection.

further information upon this point, I inoculated milk with larger amounts of tubercle bacilli in suspension, but instead of using animal tests I made smears from the different levels. Table IV shows the results of two such tests. The smears demonstrated conclusively that

TABLE IV
ANIMAL INOCULATION WITH TUBERCULOUS MILK

	BOTTLE A.	BOTTLE B.
Surface of cream....	Very large number of bacilli; some clumps.	Numerous bacilli; few clumps.
1st 2 oz.....	Few bacilli; 1 clump.	Few bacilli seen.
2d 2 oz.....	No bacilli seen.	2 bacilli seen.
3d 2 oz.....	1 bacillus seen.	1 clump seen.
Skim-milk.....	No bacilli seen.	1 bacillus seen.
Lowest 1/2 oz. (dregs).....	Few bacilli seen.	1 bacillus seen.

tubercle bacilli are also carried upward by the fat globules. It was very striking to compare the number found at the very surface of the bottle with that from even the uppermost ounce of the cream.

The statistics which I have given for gravity cream do not apply for centrifugal cream. If we centrifuge milk rapidly and for a considerable period, and then make smears from the cream, from the skim-milk, and from the sediment, we find that although many bacteria are in the upper layer, the sediment also is rich in bacteria, and contains many more than previous to the separation. If we centrifuge less rapidly or for a shorter period of time and obtain a less dense layer of cream, the conditions approach more nearly those of gravity cream. In other words, the result depends upon the relation of the artificial

centrifugal force to the natural centripetal force of gravity; if the former is very great, it will drive many of the bacteria to the sediment. I do not mean to infer by this that we are able to obtain a pure cream by means of the centrifuge, but merely that the fresh cream obtained by this method contains fewer bacteria than the gravity cream. I may also add that what I have said throughout concerning bacteria applies equally to leucocytes—that they are also carried upward by the fat and are usually found in great numbers at the surface of the cream.

Having dwelt at some length on the purely theoretical side of our subject, let us now turn to its practical application. At the present day every effort is being made to supply a milk with the lowest possible degree of contamination. The certified milk was a long stride in advance, but unhappily at the present time its cost greatly curtails its helpfulness, and the number of its beneficiaries is comparatively small. Any suggestion, therefore, which would help to procure a less contaminated milk for the masses is certainly worthy of consideration. Now that we realize that the upper two ounces of the bottle harbor the greatest number of bacteria, the removal of this portion naturally suggests itself. Of course by this procedure we lose butter fat as well as bacteria. A bottle of milk, which averages about 4.2 per cent. fat, I have found will contain but 3 per cent. when the upper two ounces have been removed. A 5 per cent. milk is reduced to a 3.25 to 3.5 per cent. milk. However, this may frequently be of advantage, as probably most of us will agree. As Holt says: "There are many healthy infants who cannot digest even 4 per cent. of fat at any time, and many more who during hot weather do much better when a reduction to 3 per cent. or 3.5 per cent. is made." It is hardly necessary for me to particularize the instances in which a 3 per cent. of milk is of value. I should like to draw attention, in passing, to the facility with which this "low-fat" milk is obtained, and to call attention to the fact that the method does not entail the necessity of pouring the milk into another vessel, and thus subjecting it to the danger of additional contamination; the cap needs merely to be reapplied to the bottle and the milk then shaken. A 3 per cent. milk procured in this manner contains fewer microorganisms than a similar milk obtained by the usual dilution of

one-fourth water, and is less apt to contain tubercle bacilli, a danger by no means to be disregarded in the ordinary bottled milk. That its volume is less than the watered milk of equal richness may also be accounted an advantage, as surely nothing is to be gained by feeding an infant with large quantities of water. When we dilute a 4 per cent. milk one-fourth by adding 11 ounces of water to a quart, we obtain a 3 per cent. milk, but at the same time reduce the proteid to 2.6 per cent. if we consider the average proteid content of cow's milk to be 3.5 per cent. On the other hand, if we use the partially skimmed milk which I suggest, although the fat likewise equals 3 per cent., the proteids remain full strength, namely, 3.5 per cent. Considering the fact that the proteids are more frequently prescribed too low than too high in later infancy, this formula should prove useful.

As a basis for the current top-milk formulæ it is customary to use a 12 per cent, or a 10 per cent. milk, and a 7 per cent, milk; in other words, one formula in which the fat content is about four or three times that of the proteid, and another where it is only twice as great. In order to bring this method into conformity with these well-established principles, I have endeavored to duplicate these percentages, and have had various portions of bottled milk tested quantitatively for fat

TABLE V.

METHOD OF OBTAINING 12 PER CENT., 10 PER CENT., OR 7 PER CENT. MILK AFTER REMOVAL OF THE UPPER TWO OUNCES

	Fat.	Proteid.
Next 7 ounces	12 per cent.	3.2 per cent.
" 8 "	10 " "	3.3 " "
" 12 "	7 " "	3.5 " "

by the Babcock method, for which tests I wish to thank Mr. Congdon, of the Chemical Laboratory of the Department of Health, and Messrs. Harrison and Jeffers, of the Walker Gordon Laboratory. Many tests were made; the results are incorporated in Table V. The upper two ounces of the bottle contain on an average 24 per cent. of fat. This estimation and those that follow are based on 4 per cent. milk, which

has been bottled about 24 hours; at any rate, for not less than 14 and not longer than 36 hours. As these two ounces are discarded, a slight variation in the fat percentage cannot result in serious error. It is true that if the milk has been bottled for 48 hours or more, this layer becomes considerably richer. After two ounces are removed, the next seven ounces contain 12 per cent. fat, 3.2 per cent. proteid; the next eight ounces 10 per cent fat and 3.3 per cent. proteid; the next 12 ounces contain 7 per cent. fat and 3.5 per cent. proteid. These figures are not absolute, as may be easily imagined; however, they compare very favorably with comparative tests made with top milk according to established methods.

I shall not weary you with an array of formulæ. The same formulæ may be prepared on this basis as are generally made use of in top milk preparations. I may add, however, that if one wishes to obtain a 4 per cent. milk with its normal quota of proteid, in other words, a full milk, one has only to add 3 ounces of the 10 per cent. cream to the 3 per cent. milk, which I have shown is so easy to prepare.

In closing, let me add that this method of preparing milk should prove particularly serviceable in the summer time, when we have so much to fear from bacterial contamination as well as from digestive disturbances due to a food too rich in fat.

My thanks are due to Dr. Park for suggestions freely given in the course of this work.

SUMMARY

In bottled milk the bacteria are by far the most numerous in the upper layers of the cream, becoming gradually fewer in its lower portion.

The upper two ounces contain the greatest number of bacteria.

This is true of tubercle bacilli as well as of streptococci and other bacteria.

Therefore, instead of using the upper cream, as is now practiced, it is preferable to discard the upper two ounces.

The average bottle of such *partially skimmed milk* contains 3 per cent. fat and 3.5 per cent. proteid and is well adapted for infant feeding.

If we discard the upper two ounces we have: next seven ounces, a

12 per cent. milk; next eight ounces, a 10 per cent. milk; next 12 ounces, a 7 per cent. milk. With these figures as a basis the usual top-milk formulæ may be prepared.

AN INEXPENSIVE HOME-MADE MILK REFRIGERATOR

ALFRED F. HESS, M.D.

Individuals and communities are at present much interested in the question of pure milk for the infants of the poor. A more rigid supervision is gradually being enforced over all those who handle milk—the farmer, the dairyman, the wholesaler, and the retailer. Although these efforts can not be too highly commended, too little stress has been laid on the importance of the care of the milk in the home of the consumer. No matter how carefully the milk has been obtained and guarded up to the time it is retailed, even if it is pasteurized or certified, it will be rendered unfit for food after standing in a room at summer heat for a few hours. That this is a real danger is known to all who have tended babies in the tenement houses in summer. Most of the people in poor circumstances have no ice or an insufficient supply, so that the milk is kept at a temperature of from 50° to 70° F.

For some months I have been endeavoring to devise a simple and inexpensive means for keeping milk in summer—one that will be within the reach of the mother in the tenement house. After considerable experimenting, I can recommend the following box for this purpose:

An ordinary packing case was obtained; it had been made for bottled water, and measured on the inside 13 by 18 inches and was 11½ inches in depth. Sufficient sawdust was placed in this box to make a substantial layer on the bottom. On this was set a tin can, tall enough to hold a quart bottle of milk and 8 inches in diameter, and around this was placed a cylinder of tin a little larger in diameter than the can. The cylinder was then surrounded by sawdust. The lid of the can was, of course, left free. The ice box was completed by nailing about 50 layers of newspapers to the lid of the case (Figs. 1 and 2). The total cost of such an apparatus is the cost of the tin can, which may be 25 or 50 cents, according to the quality. The box and sawdust can be obtained free from a grocer.

To test the value of the box, a quart of milk was placed in the can

and surrounded by 6 or 7 pounds of ice; that is to say, less than five cents worth. The room temperature was 81° F. The efficacy of the refrigerator was demonstrated by the fact that 24 hours later the temperature of the water in the can was 33° F., the milk in the bottle 37° F., and that even after 45 hours the temperature of the water had risen only to 50° F. and the milk to 52° F.

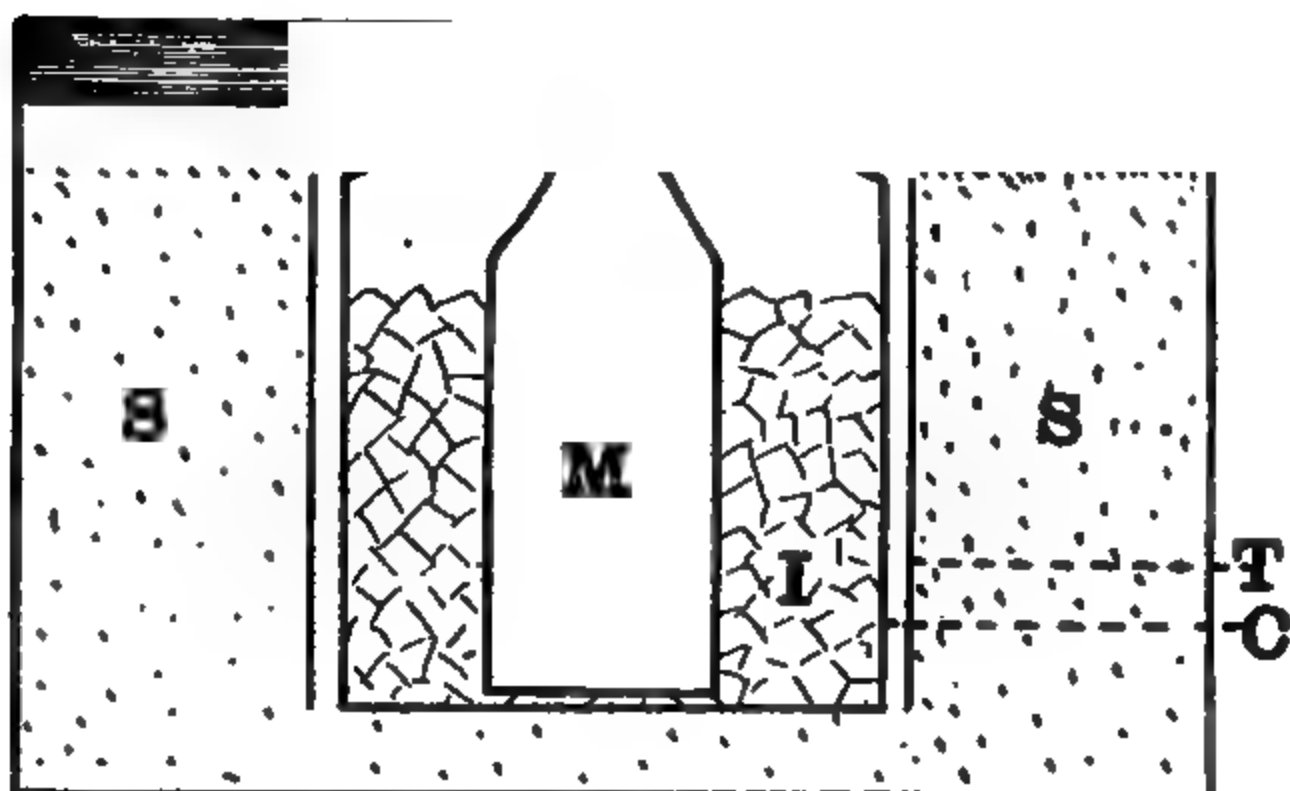


Fig. 1.—Vertical section of home-made milk refrigerator; S, sawdust, excelsior or other cheap non-conductor of heat; T, cylinder of oilcloth, tin or galvanized iron; C, can in which is placed the milk jar M, surrounded by broken ice, I; N, newspapers nailed to lid of case.

Numerous variations from this type of box were found to keep out the heat. A somewhat larger box was found more desirable. Excelsior may be substituted for sawdust. All that is necessary is that the can containing the ice be surrounded on all sides by a material which conducts heat poorly. Care should be taken that the can rests on sawdust and not directly on the wooden floor of the case. Should the case be rather shallow for the can, newspapers should be laid between the two. To prevent rusting a little soda may be placed in the can every day.

The apparatus described above will keep two quart bottles of milk, or four 8-ounce feeding bottles. The great majority of mothers in the

tenements keep the day's supply of milk in a quart bottle and possess but two or three nursing bottles. As the ideal method is to have as many bottles as there are feedings in the course of the day, it was determined to make such minor modifications in the ice box as would allow of this procedure. To this end a tin can was obtained which was $8\frac{3}{4}$ inches in diameter and cost 30 cents. It was sufficiently large to

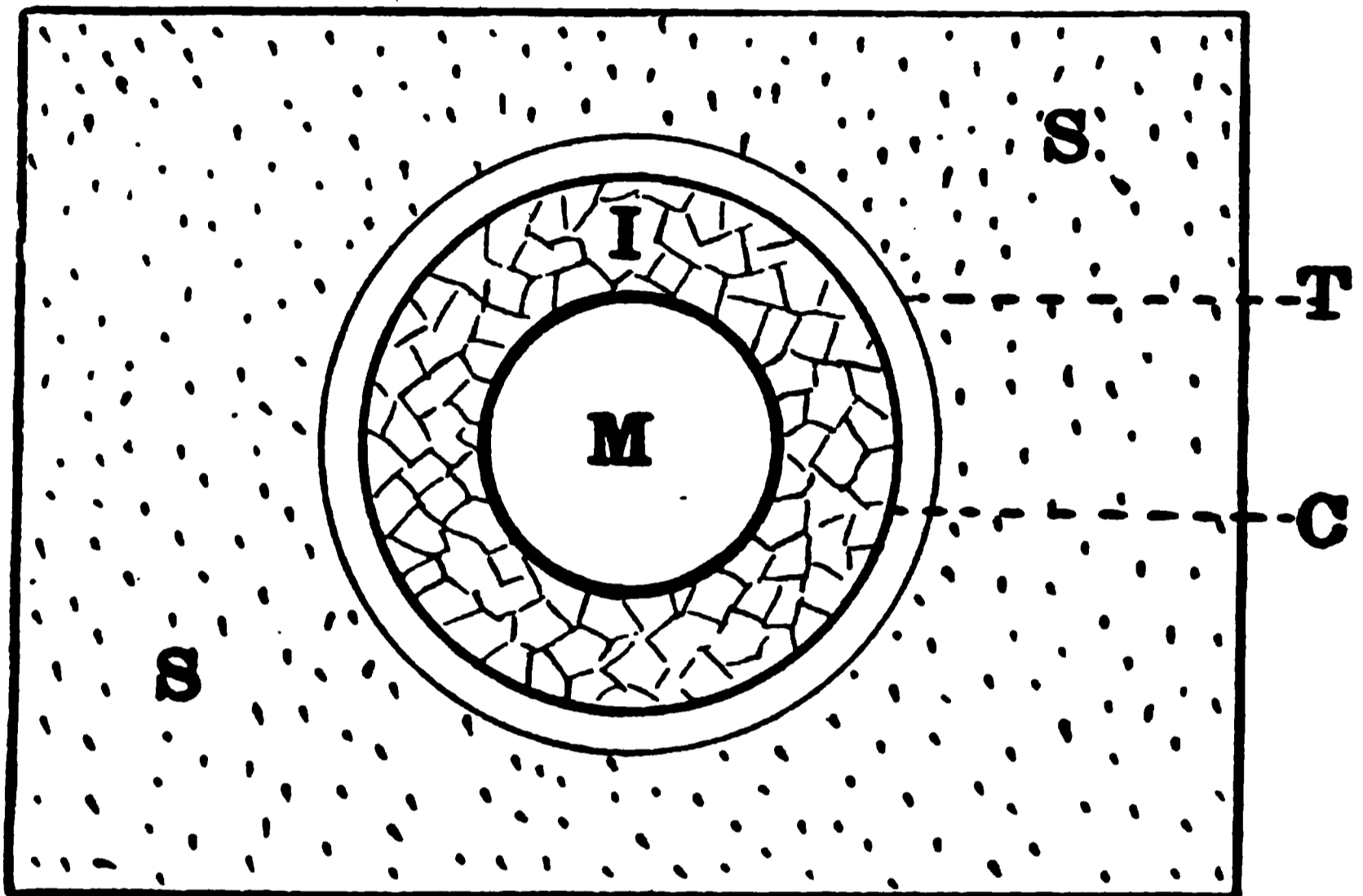


Fig. 2.—Horizontal section of home-made milk refrigerator; M, milk container; I, broken ice; C, can for holding ice; T, tin, oilcloth or galvanized iron cylinder to prevent sawdust, S, from falling into space when can is removed for purpose of emptying water.

admit a wire bottle holder costing 45 cents and containing eight bottles. A case 18 inches square was employed to hold it. The ice was cracked into smaller pieces than before, 6 or 7 pounds being used, and the wire holder with its bottles (previously cooled in running water) was then set on the surface of the ice, or rather gently pressed down into the ice. Within one hour the temperature of the milk fell from 67° to 55° F. and continued to fall. After 24 hours it was at 39° F. and the water at 38° F.

The bacterial content of the milk was 7,000 bacteria to the c.c. when it was obtained. After 24 hours the milk in the refrigerator had risen

to 42,000 to the c.c. A sample of the same milk left at a temperature of 73° F. showed 12,360,000 bacteria to the c.c.

Will mothers take the trouble to improvise ice boxes of this description? From an experience with mothers who consult the dispensaries I can say that they are anxious to do all in their power to protect their babies. Many would be quick to profit from the lesson if they saw a model of the ice box, were told how cheaply it can be constructed, how it will economize ice, and, finally, how its employment will aid in saving the baby from an attack of the much-dreaded summer complaint. The cost of such ice boxes can be considerably reduced if they are made in large quantities, so that with private and municipal co-operation they could be supplied for much less than the above figures.

Refrigerators of this design, 1½ feet square by 14 inches deep, have been distributed among the children's dispensaries, dairy kitchens, nurses' settlements and kindred organizations in New York City, where they are being demonstrated to mothers. It is hoped that this article may encourage other communities to similar action in aid of the poor babies.

A HANDBAG REFRIGERATOR FOR THE UNCONTAMINATED CARRYING OF MILK SAMPLES

CHAS. B. FITZPATRICK, M.D.

Milk samples are frequently obtained in small glass bottles holding about 30 c.c. provided with a metal screw top containing a wafer of cork as a filling. These samples are often placed in ordinary handbags, with an inside tin or zinc lining, and kept cold by being directly surrounded by ice. The ice and ice-water often completely immerse the bottles, so that they are often found floating in this ice and

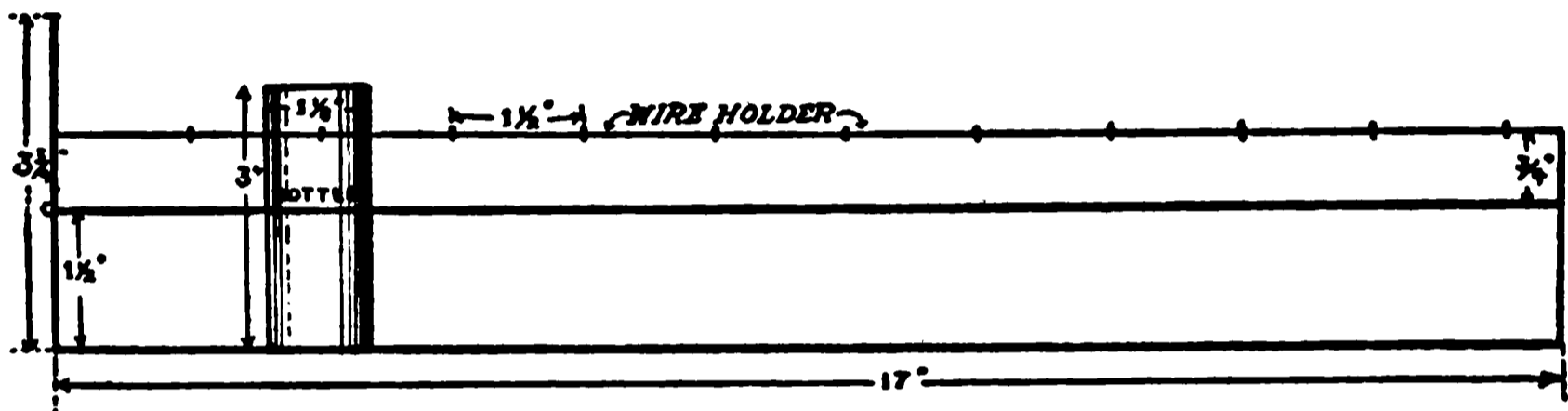


FIG. I. SIDE VIEW OF TRAY

water mixture. An examination of many of these samples showed that the bottles were not sufficiently water tight to prevent contamination of the milk samples. A simple way to avoid this source of contamina-

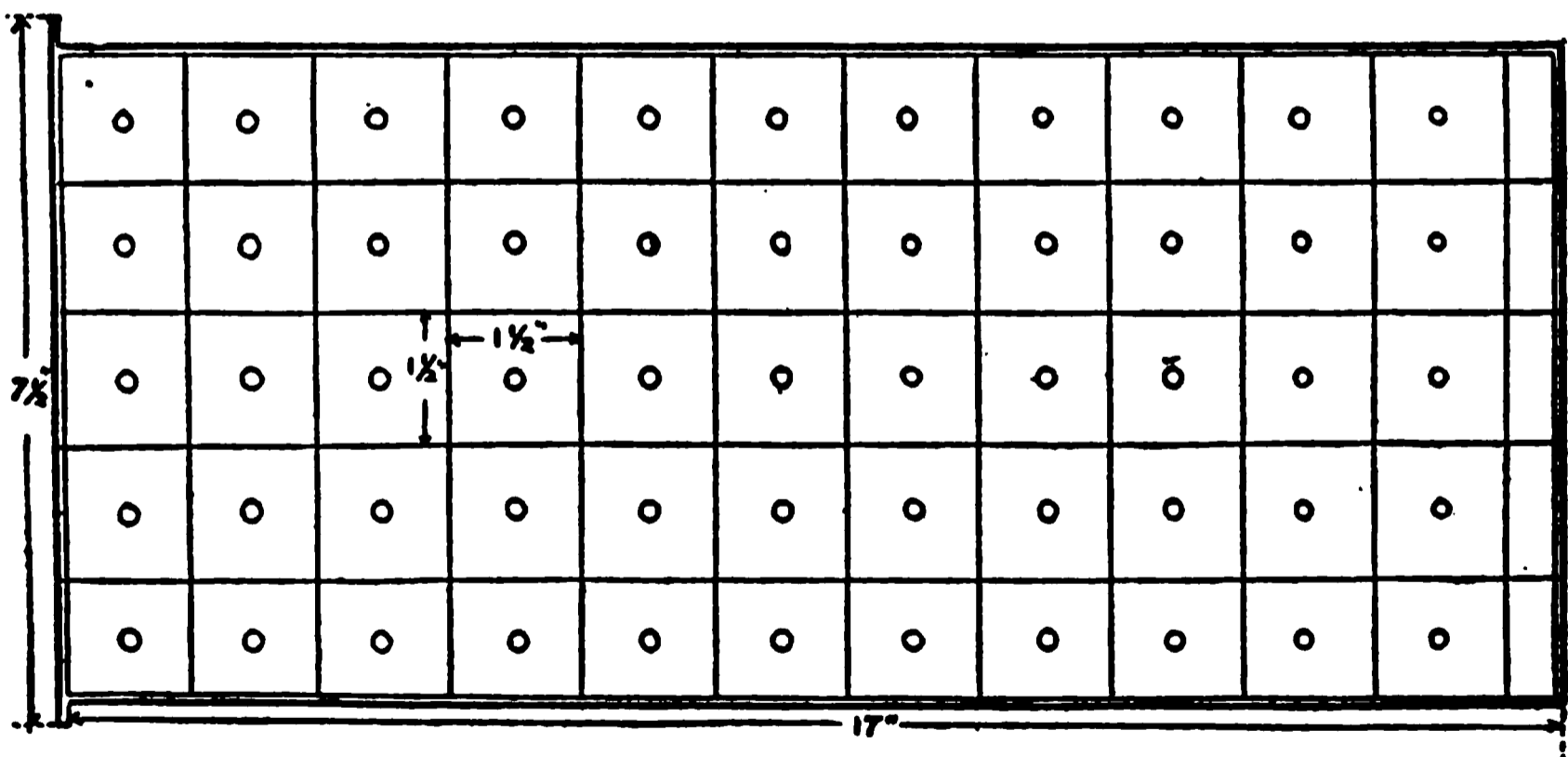


FIG. II. PLAN OF TRAY

tion is as follows: (1) Take two round tin cans 5 inches in diameter and three inches in height (sufficiently large to hold 12 of these sample bottles), which are absolutely water-tight. (2) Put a false wooden bot-

tom in the bag by means of two rods running horizontally from one end to the other of the top of this bottom which reaches the middle of the bag, supporting the two boxes and separating them from the bottom half of the bag, containing the ice. The tin cans are placed immediately above the ice and supported by the two rods. This plan avoids all contamination and the temperature is often as low as $34\frac{1}{2}^{\circ}$ F.

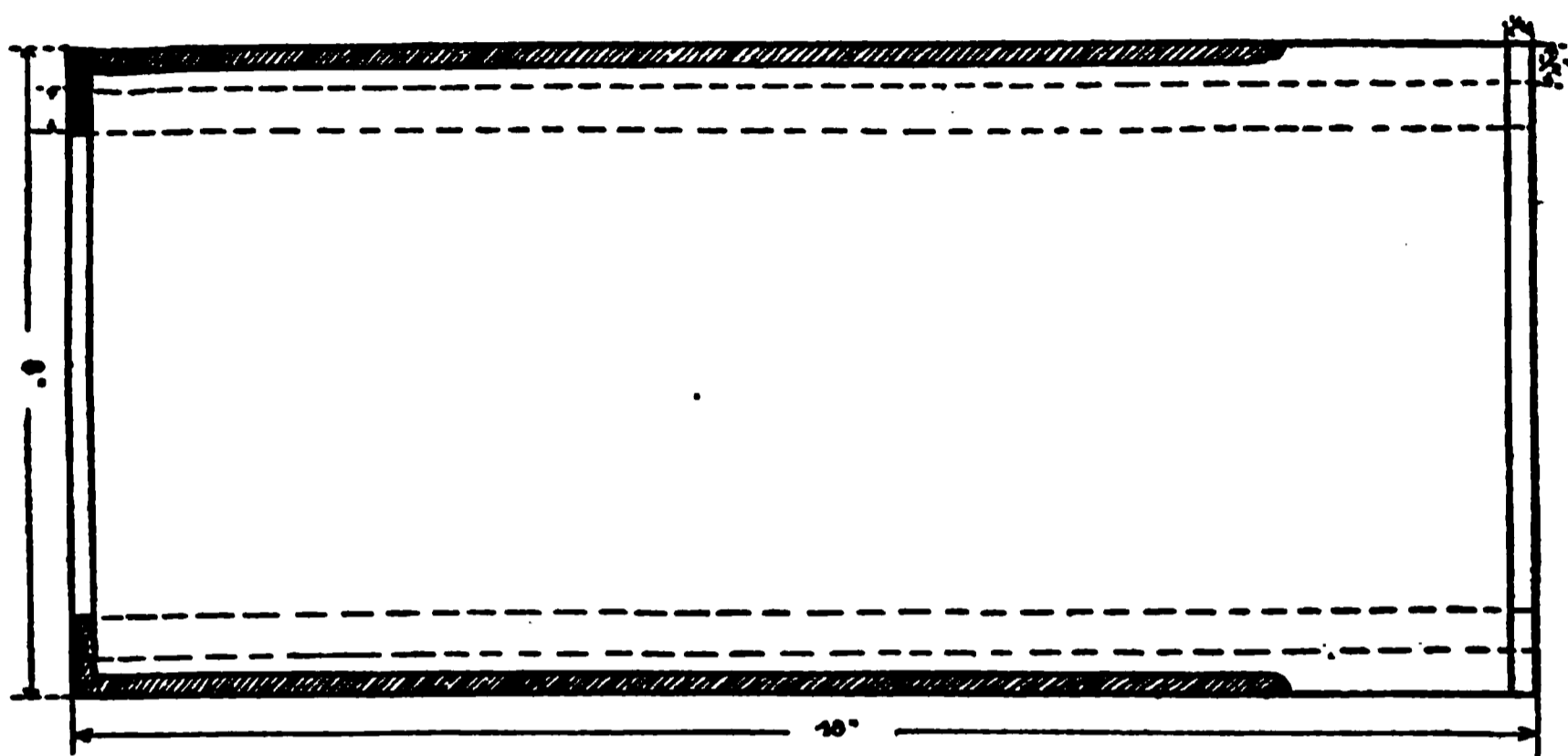


FIG. III. PLAN OF BAG

Another more elaborate way to avoid this contamination is to take an ordinary handbag and cut an opening in one end of it, so as to admit a tin drawer, approximately the length and width of the bag, abbreviated just enough to admit the drawer and to permit it to slide

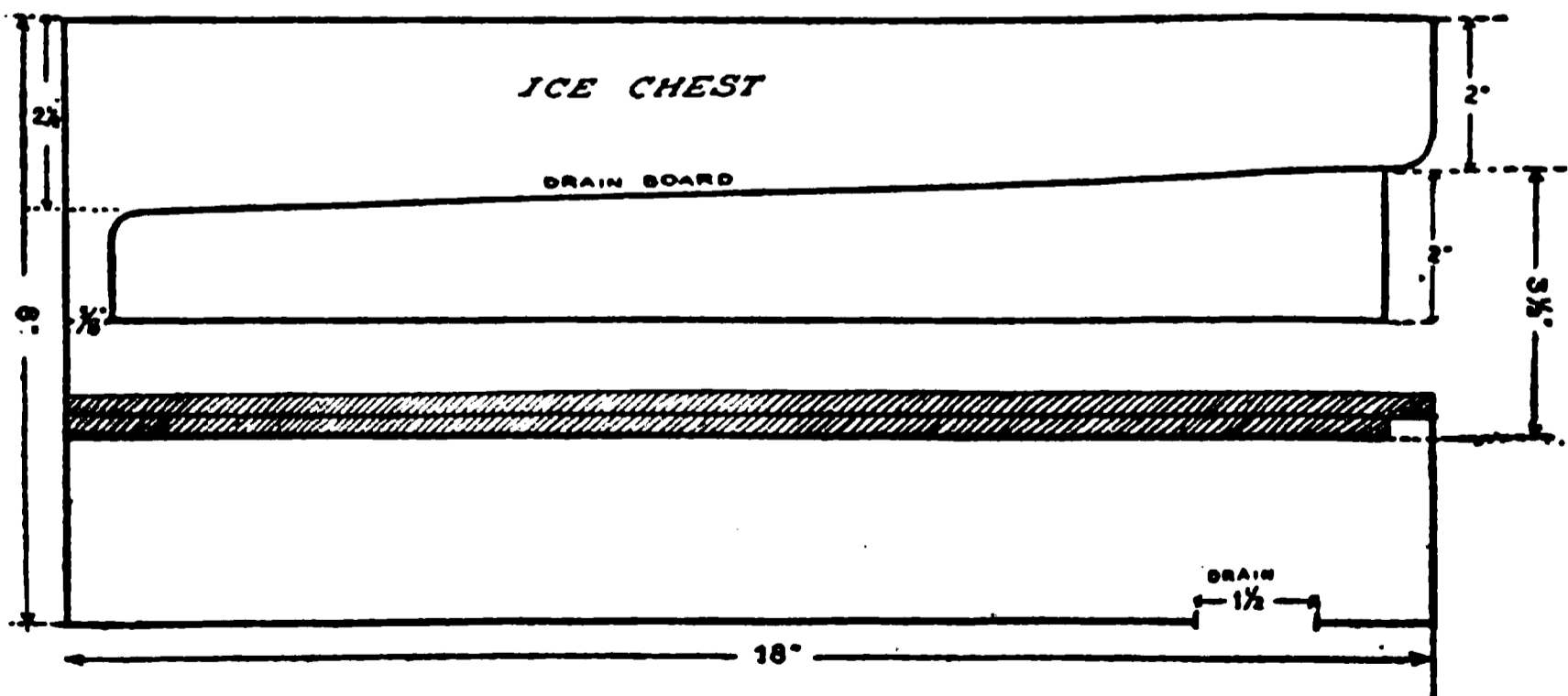


FIG. IV. SIDE VIEW OF BAG.

freely in and out. This drawer accommodates about 50 sample bottles and is completely separated from the upper half of the bag by a shelf of tin which contains the ice. This shelf is attached at the ends and

sides to the inside tin container. The long side of this roof or shelf is bent down and extends so as to cover the bottle room and hangs down at the sides to below the middle of the bottles. This overhang carries the water of the melted ice below the bottles into the tin bottom of the bag, which is 1 inch lower than the drawer and contains a screw cap outlet for the water. The bottles are thus kept dry and away from the drip. A space of 1 inch is also allowed at the side between the row

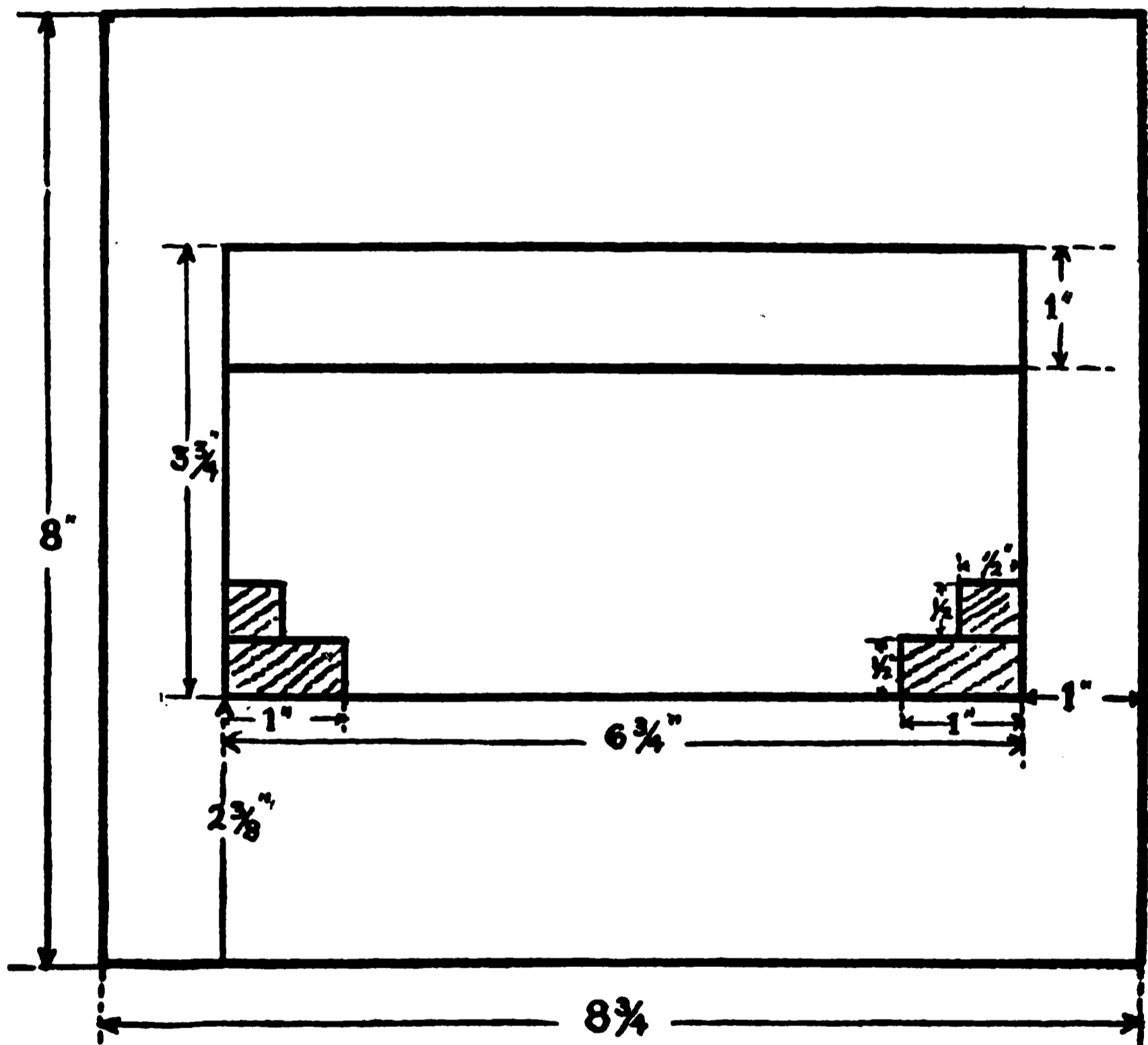


FIG. V. FRONT VIEW OF BAG

of bottles and the side overhang. One end of this shelf connects directly with the inside tin container. The bottom of the drawer contains fine perforations. The temperature in this bag, when ice is used, averages about 36° F. (Fig. I to VI incl.).

Various modifications may be made. In order to see how cheaply an efficient bag could be improvised, the two tin cans were taken and one was filled with ice while the other contained the samples. The one

containing the ice was placed directly upon the top of the one holding the samples. A felt bottom was attached to this and another piece of felt was wrapped around it, thus providing sides. A felt cover was put on this and a piece of wire wound twice around the sides, the bottom and top were likewise held in place by two bands of wire placed at right angles, and a wire handle improvised. This made an efficient refrigerator that looked like an ordinary pail, and which could be made out of any pliable material that was a good non-conductor of heat.

This two separate containers idea may be also applied to the bag by simply making the shelf which divides the bag in two connect directly to all the sides, thus making two distinct non-communicating compartments. The drawer fits in as before described.

FIG. VI. SKETCH OF TRAY

A further improvement readily suggests itself, namely, to make a bag of one-half (or less) the length of the bag (Fig. I to VI incl.), designed to carry 25 (or less) bottles. The trays, each holding about 12 bottles, could also be arranged, so as to be detachable (together with the bottom of the bag), thus enabling one to have as small a bag as possible to carry for each occasion. I desire to thank Mr. Russell Raynor, Chief of the Divisions of Inspections, for his kindness in helping me to have this work finished. The ready co-operation of Milk Inspector Walters and Ass't Chemist Alexander is also acknowledged.

THE PRESENCE OF TUBERCLE BACILLI IN THE CIRCULATING BLOOD

BY

CHARLES KRUMWIEDE, JR.

That tubercle bacilli could be demonstrated in the circulating blood or in the blood obtained at autopsy, in a small number of cases has long been an accepted fact.¹ Recently Rosenberger² has examined the blood in 125 cases of tuberculosis and reported positive results in all. These cases embraced every degree of severity from slight infection to the acute miliary type. If tubercle bacilli are regularly present in the blood, and can be demonstrated with such certainty, Rosenberger's communication would be an important advance in methods of diagnosis. For this reason we have tried a small number of cases of frank pulmonary tuberculosis, most of them well advanced with numerous tubercle bacilli in the sputum.

The technique employed was that suggested by Rosenberger. The test tubes, pipettes and slides were all new, except in the first two cases, where stock pipettes were used.

Four cases and two controls were on the service of Dr. Nathaniel B. Potter at the French Hospital; five cases were from the Riverside Hospital, making a total of nine cases.

Besides the examination of slides, some of the sediment was spread over the surface of glycerin egg and the remainder injected into guinea pigs.

The microscopical examination was negative in all cases except one. In this case there were acid fast clumps, resembling tubercle bacilli, but not typical. They could be found in only two slides. Other slides from the sediment were completely negative. Blood was again taken from this case. Both samples were completely negative. Stock pipettes* were used in the first sample, new pipettes in the second and

¹Bacteriologie des Blutes bei Infektionskrankheiten. P. Canon (Fischer-Jena, 1905), p. 135.

²American Journal of Medical Sciences, February, 1909.

* These pipettes, however, had never been used on tuberculous material.

third. This was the result in examining three slides from a case, each slide being examined one-half hour. Subsequently these slides were re-examined by Dr. G. B. Foster spending much more time on the individual slides, in some instances three hours, with the following results: One control case, clinically secondary syphilis with no suspicion of tuberculosis showed three clumps of eight to thirty bacilli. The duplicate slides from the case showing enormous numbers were found negative. In four of the cases of pulmonary tuberculosis which had been negative, one showed two small clumps and a few isolated bacilli, two showed a few isolated bacilli and one a few intracellular acid fast bodies. These isolated rods had been observed in the first examination but were thought not to resemble tubercle bacilli sufficiently to call the slide positive.

The attempts at cultivating tubercle bacilli from the sediment were negative in all cases.

As to the inoculations into guinea pigs, in two cases the pigs died acutely. The pig receiving the sediment which apparently gave a positive microscopic examination died in 21 days and was negative. The second sample from this case proved negative in pigs; the third died acutely. The pigs of the remaining six cases were completely negative.

After the above results we feel that the finding of isolated bacilli in blood smears of supposed tubercular patients would be a precarious method of diagnosis. The finding of clumps of bacilli raises the question, why, if they were so numerous as in one case, were not subsequent examinations also positive and why were culture and guinea pig inoculations negative? Further, the same acid fast bacilli were found in a case which had not the slightest suspicion of tuberculous infection. The argument that in the first case the bacilli were dead and only discharged into the blood at the time the examination was positive and that in the second case there was an undiscovered tuberculous lesion is slightly incredible.

We conclude that acid fast bacilli may be found, but that these findings are valueless in diagnosis. In view of the discordant results we feel that contamination from water or air is the most likely source of these acid fast bacilli, though we have no proof of this.

NOTES ON THE EXPERIMENTAL PRODUCTION OF FAGET'S DIAGNOSTIC REACTION OF YELLOW FEVER

BY CHARLES B. FITZPATRICK, M.D.

Faget, from his studies of the New Orleans (1870) and the Memphis (1873) epidemics, discovered that a falling pulse with a rising or horizontal temperature is a diagnostic reaction, pathognomonic of yellow fever. His conclusions are widely followed in the South, and this reaction is considered by many physicians to be more or less pathognomonic of this disease.¹

Hence, I have been led to briefly outline some results obtained by injecting the toxic contents of bacteria isolated from cases of yellow fever. The culture which furnished most of the reactions was isolated by me from the liver of a case of yellow fever and named *B. coli icteroides*.² The other culture was one of the *B. icteroides*, sent to me by Sanarelli; both of these cultures belong to the colon group. The *B. coli icteroides* was first tested on dogs. It was prepared for this purpose by being incubated for 24 hours in bouillon at 37° C., then for 24 hours at 40° C., and then left for 24 hours between 45° and 50° C., and finally was exposed for five minutes at 55° C. One dose of 15 cubic centimeters was injected subcutaneously into a healthy dog weighing 45 pounds. When the reaction had completely disappeared the same dog was inoculated with a fatal dose of the *living* culture of the *B. coli icteroides*. The dog recovered after a severe and protracted reaction. A control dog was inoculated at the same time with an equal amount of this living culture and it died in about 24 hours. This procedure was repeated with the *b. icteroides* and the same results were obtained. Another culture of the *b. coli icteroides*, slightly different from the one already employed, was similarly investigated and gave like results. These three cultures were then mixed together in equal parts, treated as the single culture and gave the same results. Finally, a sterile fluid was prepared of these three cultures, which had been

¹ J. C. Faget, *Yellow Fever*, pp. 1-49, J. B. Balliere & Sons, Paris.

² Fitzpatrick, *N. Y. Medical Record*, Jan. 29, 1898, and June 1, 1899.

grown for four days, then killed by exposure and heat and further disintegrated.

The use of this preparation in humans has given some interesting results. Eleven of the persons who were injected subcutaneously with from one-half of a c.c. to 5 c.c. reacted with the characteristic falling pulse and a rising or horizontal temperature, which is known as Faget's reaction of yellow fever. Chill, headache, malaise and pain at the point of inoculation were also noted.

CHART OF ELEVEN FAGET'S DIAGNOSTIC REACTIONS OF YELLOW FEVER
(i.e. Falling Pulse with Rising or Horizontal Temperature)
Experimentally produced in man by Subcutaneous Injections of Toxins obtained
from *B. coli commune* (*B. coli icteroides* and *B. icteroides*)

1* (1 c.c.)	2* (3 c.c.)	3† (½ c.c.)	4† (2 c.c.)	5 (1 c.c.)	6 (1 c.c.)	7 102° (2 c.c.)	8 (2 ½ c.c.)	9 (2 c.c.)	10 (1 ½ c.c.)	11 (2 c.c.)
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*Reactions 1 and 2 belong to one case.
†Reactions 3 and 4 also belong to one case.
The other reactions are each from separate cases.

Each No. (2 to 11) on this line indicates a separate reaction. The amount of subcutaneous injection is within the () immediately following the number.

EXPERIMENTAL DATA RELATING TO HAEMOLYTIC SERA

BY

L. W. FAMULENER, M.D., and ALICE G. MANN

GENERAL OUTLINE

- A. NATURAL IMMUNE BODIES IN NORMAL SERA
 - I. Action of Normal Sera from Different Species upon Alien Blood Cells.
 - II. Action of Normal Sera from Different Individuals of the Same Species upon Alien Blood Cells.
 - III. Action of Natural Immune Body Influenced by an Alien Complement.
 - IV. Action of Natural Immune Serum upon Blood Cells of Different Individuals of the Same Species.
- B. COMPLEMENTING SERA
 - I. Comparative Complementing Values of Rabbit, Sheep and Goat Sera with a Specific Immune Serum.
 - II. Comparative Complementing Values of Guinea-pig Serum and Rabbit Serum with a Specific Immune Serum.
 - III. Comparative Complementing Values of Guinea-pig Serum and Rabbit Serum when Acting upon Homologous Specific Immune Sera from Alien Species of Animals.
 - IV. A Comparison of Sera from Normal Rabbits and Sera from "Snuffle" Rabbits as to Their Relative Complementing Action.
- C. HAEMOLYSIS AS AFFECTED BY CONCENTRATION OF DIFFERENT COMPONENTS OF THE IMMUNE BODY—COMPLEMENT COMPLEX
 - I. Constant Amount of Complement, Constant Volume of Fluid, Variable Number of Blood Cells and Decreasing Amounts of Immune Body.
 - II. Constant Amount of Complement, Variable Volume of Fluid, Constant Number (approx.) of Blood Cells and Decreasing Amounts of Immune Body.

CONCLUDING REMARKS.

INTRODUCTORY REMARKS

Perhaps no group of experimental studies has had greater influence upon the general problem of immunity than that involving haemolytic sera and their reactions. These studies have not only embraced the immune body complement complex, but have been extended to include definite haemolytic toxins of bacterial origin and their specific antibodies. In many respects these bacterial toxins and antitoxins are analogous to those which hold such an important place in medicine. But their interactions can be studied with greater ease and probably with a greater degree of accuracy since the test tube method of investigation is applicable. By such a method the investigator has a means of controlling various factors and conditions of experimentation at his will, and is able to read the results with mathematical precision. It is quite obvious that a means of study is afforded which approaches the

ideal—a condition far from attainable in purely animal experimentation.

The study of haemolytic sera has thrown much light upon the nature of cytolytic substances in the body fluids. Moreover the subject has been found to possess a practical application in other fields. Noteworthy in this connection may be mentioned the tests for blood stains (Deutsch, and the Neisser-Sachs) in medico-legal cases; the Bordet-Gengou reaction (fixation of complement), for the recognition of a large class of specific antibodies; the Wassermann test and its various modifications in the diagnosis of syphilis; investigations in connection with the cancer problem; the study and differentiation of micro-organisms into sub-groups; the examination for the presence of normal haemolysins prior to transfusions, etc.

Although an extensive literature has developed in connection with this subject, many of the basic data are fragmentary, and so widely dispersed throughout the whole that they are hardly accessible. It is often necessary for the individual worker to do much preliminary work in order to establish data before undertaking a definite problem involving these reactions. Since the writers have had to cover this ground, preliminary to certain haemolytic studies, it was deemed worth while to collect some of their data and make them accessible for future reference. In some cases additional work has been done for the purpose of further completeness, although no attempt has been made to cover all conditions. The subject matter to follow will include certain tests which may throw light upon particular experimental conditions, or may act as a check to prevent errors in technic. The protocol tables will be given intact in some cases; in other instances various steps will be tabulated in order to indicate the development of the method used; some results will be shown by diagrams, while others will be represented graphically by curves. Explanatory notes will be given in connection with each series of tests.

This work will be confined to those reactions in which haemolytic sera enter, and will include immune body and complement relations. It is taken for granted that laboratory workers in this particular line have an understanding of the fundamental theories pertaining to the interaction between cells, immune body and complement. Such information

may be derived from recent text-books on bacteriology, pathology, or special works on the subject of immunity.

A. NATURAL IMMUNE BODIES IN NORMAL SERA

I—Action of Normal Sera from Different Species upon Alien Blood Cells

It is a well-known fact that the blood serum of one species of animal may dissolve the blood cells of an animal of another species; that is, possesses a haemolytic action for those particular blood cells. While on the other hand, the serum of like species does not dissolve the homologous blood cells, except under certain abnormal conditions. But the serum of the alien species does not always possess this haemolytic action; in fact, it may be quite inert.

Since the discovery of natural immune bodies (haemolytic), and complement in fresh normal serum, an explanation is given for such phenomena. Haemolysis only takes place after the particular blood cells have become sensitized by the absorption of immune body, and then through this combination become subject to the action of complement. In the absence of either specific immune body or complement haemolysis does not take place.

The appended table (No. 1), taken from our tests, offers an excellent illustration of how, in some cases, the presence of natural immune bodies acts against alien blood cells. It also shows how such bodies in a single serum may act upon the blood cells of several species of animals. Dog serum, for example, haemolysed the blood cells of horse, goat, sheep, rabbit and guinea pig.

TABLE I

NATURAL HAEMOLYSIS IN NORMAL SERUM

Key:—

Comp. H. = Complete solution (haemolysis) of blood cells.
++++ = Almost complete haemolysis of blood cells.
++ = Partial haemolysis of blood cells.
+ = Definite coloration of supernatant fluid.

Sl. + = Lesser coloration of supernatant fluid.
t = Only a tint in supernatant fluid.
Sl. t. = Slight tint in supernatant fluid.
o = No coloration in the supernatant fluid—water white.

Dog Serum.	*5% Susp. of Blood Cells.					Horse Serum.	5% Susp. of Blood Cells.					Goat Serum.	5% Susp. of Blood Cells.				
	Horse.	Goat.	Sheep.	Rabbit.	†G. P.		Goat.	Sheep.	Rabbit.	G. P.	Dog.		Sheep.	Rabbit.	G. P.	Dog.	Horse.
1.00 c.c.	+	+	+	+	Comp. H	1.00 c.c.	o	o	+	t.	o	1.00 c.c.	o	o	Comp. H	o	+
0.80 "	+	+	+	+	"	0.80 "	o	o	+	sl.t.	o	0.80 "	o	o	"	o	+
0.60 "	+	+	+	+	"	0.60 "	o	o	+	o	o	0.60 "	o	o	"	o	+
0.50 "	+	+	+	+	"	0.50 "	o	o	t.	o	o	0.50 "	o	o	"	o	+
0.40 "	+	+	+	+	"	0.40 "	o	o	t.	o	o	0.40 "	o	o	"	o	t.
0.30 "	+	+	+	+	"	0.30 "	o	o	sl.t.	o	o	0.30 "	o	o	+	+	sl.t.
0.25 "	+	+	+	+	"	0.25 "	o	o	"	o	o	0.25 "	o	o	+	+	"
0.20 "	+	t.	+	+	+	0.20 "	o	o	o	o	o	0.20 "	o	o	+	t.	o
0.15 "	+	o	t.	.	+	0.15 "	o	o	o	o	o	0.15 "	o	o	t.	o	o
0.10 "	+	o	o	o	+	0.10 "	o	o	o	o	o	0.10 "	o	o	o	o	o

Sheep Serum.	5% Susp. of Blood Cells.					Rabbit Serum.	5% Susp. of Blood Cells.					Guinea Pig Serum.	5% Susp. of Blood Cells.				
	Horse.	Goat.	Dog.	Rabbit.	G. P.		Goat.	Sheep.	Horse.	G. P.	Dog.		Sheep.	Rabbit.	Goat.	Dog.	Horse.
1.00 c.c.	++	o	o	++	Comp. H	1.00 c.c.	t.	t.	Comp. H	o	t.	sl.+	sl.+	t.	sl.+		
0.80 "	++	o	o	++	"	0.80 "	t.	o	"	o	t.	"	t.	t.	t.		
0.60 "	+	o	o	++	"	0.60 "	sl.t.	o	"	o	sl.t.	t.	o	t.	o		
0.50 "	+	o	o	++	"	0.50 "	o	o	"	o	o	o	o	o	o		
0.40 "	t.	o	o	++	"	0.40 "	o	o	++	o	o	o	o	o	o		
0.30 "	sl.t.	o	o	+	"	0.30 "	o	o	++	o	o	o	o	o	o		
0.25 "	o	o	o	sl.t.	"	0.25 "	o	o	++	o	o	o	o	o	o		
0.20 "	o	o	o	o	++	0.20 "	o	o	++	o	o	o	o	o	o		
0.15 "	o	o	o	o	++	0.15 "	o	o	++	o	o	o	o	o	o		
0.10 "	o	o	o	o	+	0.10 "	o	o	sl.t.	o	o	o	o	o	o		

* Final mixture of serum, salt sol. (0.9%), and blood cells, gave 3.0 c.c. of 5% suspension of corpuscles.

† Guinea Pig.

The tests recorded in this table were made by selecting six of the more available laboratory animals and drawing sufficient blood from either the jugular vein or the carotid artery into a sterile flask containing a spiral of medium sized wire. The blood was defibrinated at once by vigorously shaking the flask, the fibrin for the most part collecting about the wire. The whole blood was poured off into large centrifuge tubes, and the cells separated from the serum by centrifuging. Then the supernatant serum was removed by pipette, put in bottles, and placed into the ice box until ready for use. To the corpuscles remaining in the tube several times their volume of 0.9 per cent. NaCl was added, and the whole well shaken, again centrifuged, the supernatant fluid from the blood cells removed, then the washing procedure repeated several times, until the cells were practically free from any appreciable amount of serum. After the last washing, and removal of supernatant fluid, the well packed corpuscles were drawn off by pipette and measured. Just before the blood cells were to be used in the experiment, a $7\frac{1}{2}$ per cent. suspension was prepared in 0.9 per cent. NaCl solution.

Suitable test tubes were placed in holders in series of ten each, and into these was pipetted the fresh serum (containing complement and natural immune bodies) in decreasing amounts as indicated in columns under "——— Serum" in table. Sufficient 0.9 per cent NaCl solution was added to bring the volume of fluid up to 1.0 c.c. in each tube, then 2.0 c.c. of the blood cell suspension ($7\frac{1}{2}$ per cent. corpuscles) were added to each and the whole well shaken at once. The resulting mixtures in test tubes were equivalent to 3.0 c.c. of 5 per cent. blood cell suspension, with decreasing amounts of complement and immune body. The tubes were placed in the incubator at 37° C. for one hour, then removed, thoroughly shaken again, and placed in the ice box. After 12 to 14 hours the corpuscles which were not haemolysed settled to the bottom of the tube. When haemolysis had taken place the supernatant fluid showed different degrees of red coloration or "laking." Readings were made of the haemolysis by recording the amount of "laking" roughly indicated by the marks given with the table (see key). The result of the tests may be briefly summarized as follows:

1. Dog serum; shows a marked hæmolytic action upon the blood cells of the horse, goat, sheep, rabbit and guinea pig.

2. Horse serum; no hæmolytic action upon the blood cells of goat, sheep and dog; a slight action upon guinea pig corpuscles, while rabbit corpuscles were moderately hæmolyzed.

3. Goat serum; marked hæmolytic action upon rabbit, horse and guinea pig corpuscles; no action upon blood cells of sheep or dog.

4. Sheep serum; marked hæmolytic action upon guinea pig blood cells; somewhat less upon those of rabbit, and moderate action upon horse corpuscles. No action upon goat or dog corpuscles.

5. Rabbit serum; marked action upon horse and guinea pig corpuscles; very slight action upon goat and sheep blood cells. No action upon dog blood cells.

6. Guinea pig serum; only a slight hæmolytic action upon the blood cells of sheep, rabbit, goat, dog and horse.

It is noteworthy that in the above tests in no case did the alien serum hæmolyse the dog corpuscles, while the dog's serum hæmolyzed all the alien blood cells. Since only one dog was used, it is possible that these results were peculiar in this case. Its serum was opalescent, containing a fat-like substance.

In these special tests the immune bodies were activated only by the amount of complement present in the serum under examination; that is, the fresh serum was used without the addition of any other complementing serum. As is evident, the introduction of an alien serum for complement would have greatly complicated or even invalidated certain of the tests. But as will be demonstrated later, the amount of complement present in a fresh serum may be quite insufficient to fully activate all of the natural immune body present. In such case the complete hæmolytic power is not brought out in such tests.

Certain of the tests forming parts of the table were repeated with similar results each time. However, in other cases only one test was made, notably in the case of the dog serum, and dog blood cells in relation to the other sera and bloods. Exceptional cases have been reported where the sera from individuals of like species varied greatly in action upon the blood cells of an individual of an alien species—the serum of

one animal might produce marked haemolysis, while that from a second would be without action. The same variation has been found in the resistance of blood cells, from different members of the same species, to the lytic action of a particular serum of another species. We shall have occasion to take up this point more fully later. These facts will account for the discordant reports which occur in the literature pertaining to natural haemolysins.

In practical work where a specific haemolytic serum is being used, it is important to select for complement a serum which has no natural immune body for the specific blood cells used in the experiment. Also, for most cases, it is better to select blood cells for immunizing purposes which are not normally haemolysed by the fresh serum of the animal to be treated. The futility of the opposite procedure becomes evident in cases, where, for example, transmitted immunity from mother to offspring is under investigation. In this connection the table submitted may be of value as a reference guide, but, as pointed out above, the sera of individuals vary in their haemolytic properties; likewise blood cells are more or less variable in resistance, so preliminary control tests should always be made on animals selected for haemolytic studies.

II—Action of Normal Sera from Different Individuals of the Same Species upon Alien Blood Cells

In order to compare the relative values of natural immune bodies in sera from a given species of animals, five normal goats were bled, the blood defibrinated, and the serum removed. The fresh sera thus prepared were added, in decreasing amounts, to series of tubes containing a given amount of horse corpuscles from a normal animal. The final mixture of goat serum, blood suspension and physiological salt solution gave 3 c.c. volume, containing 5 per cent. suspension of blood cells. This general technic was followed throughout the entire series of experiments. The mixture in the tubes was well shaken, placed in incubator at 37° C. for one hour, then removed and again shaken, placed in ice box until the whole cells settled to the bottom of the tubes, leaving a supernatant transparent fluid above. The first column in Table II indicates the amount of serum used; the following

columns indicate the different goats and the results of the test. In examining the table it will be observed that the serum of no two animals showed equal amounts of immune body, lytic to the red blood cells of the horse. Under goat "A" the striking fact is brought out that certain animals may be an exception to the general rule that goat serum haemolyses horse blood cells. Apparently all graduations of lytic power may exist. The serum of goat "D" showed only slight haemolytic action, while that of "B," "E" and "C" showed an increasing strength in the respective order given.

It must be remembered that in this experiment we are not dealing with natural immune body alone, but also with complement. The fresh serum while containing both of the active haemolytic elements may not contain sufficient complement to activate all of the immune body which is present. Further, the complement of that same animal which is furnishing the immune body is not necessarily the most active one (for that particular immune body) which may be had. Therefore, in this experiment it must be understood that these factors may, and at times certainly do enter, thus altering the real values. But, the test serves to demonstrate that the amount of a particular natural immune body in the serum of adult individuals of the same species is not constant, even when the animals have been kept under the same conditions, etc.

TABLE II

VARIABLE HAEMOLYTIC ACTION OF SERA FROM NORMAL GOATS UPON HORSE BLOOD CELLS

Amount Goat Serum.	Goat "A."	Goat "B."	Goat "C."	Goat "D."	Goat "E."
1.00 c.c.	o	++	+++	sl.+	+++
0.80 "	o	+	+++	"	+++
0.50 "	o	+	++	t.	++
0.30 "	o	sl.+	++	sl.t.	++
0.20 "	o	t.	++	o	t.
0.10 "	o	o	++	o	sl.t.
0.05 "	o	o	sl.+	o	o
0.02 "	o	o	o	o	o

III—Action of Natural Immune Bodies as Influenced by an Alien Complement

As pointed out above, it is questionable if a fresh serum containing both natural immune body and complement ever contains sufficient of

the latter to activate the entire content of immune body. This point was tested by drawing blood from a normal goat, defibrinating and removing the serum after centrifuging. The serum was divided into two portions; one portion was placed in the ice box until ready for use; the other was "inactivated" by heating at 56° C. for 30 minutes in a water bath. The heating destroys the complementing action of the serum without appreciably affecting the immune body. A normal guinea pig was placed under slight anaesthesia (ether), then bled to death from the carotids. The blood was defibrinated at once, and the serum was removed from the blood cells after separation by centrifuging. This serum was used in amount of 0.10 c.c. per tube as additional complement in certain series of the experiment. In control tests it was found that fivefold this amount of guinea pig serum exercised no haemolytic action upon the blood cells of the horse which supplied the corpuscles for the test. By consulting Table III the amounts of goat serum used in each series will be found recorded in the left hand column. In column "I" only fresh or "active" goat serum was added (in decreasing amounts) to series of tubes to which were added 2 c.c. of 7½ per cent. suspension of horse blood, then sufficient physiological salt solution to bring total volume to 3 c.c., or equivalent to a 5 per cent. blood suspension; column "II," the same, with the exception of 0.10 c.c. guinea pig serum for additional complement; column "III," the heated or "inactivated" goat serum was used, quite the same as in series "I"; while in column "IV" the "inactivated" serum was added as in series "III," with the addition of 0.10 c.c. guinea pig serum from complement.

The results as indicated in the table (III) show that the natural immune body of goat serum, in this case at least, when activated by its own complement, possessed marked haemolytic action (I). But when additional complement was added (II), the haemolytic action was increased, that is, in the first series more immune body was present than necessary for the amount of native complement present, so that the full haemolytic power was not brought forth in that case. In the series "III" where no "active" complement was present, naturally no haemolysis took place. However, in the last series, "IV," when 0.10 c.c. guinea pig serum was added for complementing action to the "in-

activated" goat serum, the haemolytic power was practically restored, about equal to that which originally existed in the fresh goat serum.

We see, therefore, that haemolytic sera may depend very much upon

TABLE III
INFLUENCE OF ADDITIONAL ALIEN COMPLEMENT UPON NATURAL IMMUNE BODY IN
GOAT SERUM, ACTING UPON HORSE BLOOD CELLS

Amount Goat Serum	I. Active Goat Serum.	II. Active Goat Serum + 0.1 c.c. Comp.	III. Inactive Goat Serum.	IV. Inact. Goat Serum + 0.1 c.c. Comp.
0.20 c.c.	+	++	o	+
0.15 "	+	++	o	+
0.10 "	t.	++	o	t.
0.08 "	sl.t.	+	o	t.
0.06 "	"	+	o	sl.t.
0.05 "	v.sl.t.	t.	o	"
0.04 "	"	sl.t.	o	v.sl.t.
0.03 "	o	v.sl.t.	o	o
0.02 "	o	"	o	o
0.01 "	o	"	o	o

the kind and amount of complementing serum present for their maximum action. These points will be more fully developed later.

IV—Action of Natural Immune Serum Upon Blood Cells of Different Individuals of the Same Species

Previously it has been pointed out that different sera of the same species of animal possess variable haemolytic action upon the corpuscles of another animal. It might be of interest to know whether the corpuscles of certain individuals possess relatively greater or less resistance to haemolysis than other members of the same species. For this purpose bleedings were made from six different horses which had been immuned against diphtheria toxin—the only horses available. Obviously the use of such animals' blood for this test is open to objection, still on the whole all had undergone like treatment. If the injections of toxins altered the blood cells to any appreciable extent, this change would be as a "constant," running throughout all of the animals. Apparently any marked individual difference in blood cells would show in the tests. After the blood was drawn, defibrinated and serum re-

moved, the blood cells were vigorously washed in 0.9 per cent. NaCl solution to remove all traces of free serum or antitoxin. The technic of the tests was carried out as outlined previously. The immune body was derived from blood serum of normal goat—a natural immune body. Table IV (a) will make the experiment clear, while the diagram IV(b), showing a comparative colorimetric scheme which practically represents the table in skeleton form. The schematic curve, however, gives a better comparison of the lytic variability existing between the different individuals' corpuscles than is brought out in the table. It was obtained by making a colorimetric comparison throughout the different series of horse bloods used. For example, the third tube in the first series (horse No. 323), which contained 0.5 c.c. of the goat serum, was taken as an arbitrary "standard color" tube; it showed a definite degree of coloration due to the haemolysis which had taken place in it. This "standard" was matched with those in the second series (horse No. 338), and it was found that the second tube (containing 0.8 c.c. goat serum) in that series corresponded with the standard color tube; it was so marked in the diagram. When compared with the tube in the third series (horse No. 343) it was found that the color of the third tube (0.5 c.c. goat serum) was considerably less than the "standard," while the second tube (0.8 c.c. goat serum) was somewhat greater; but the standard color closely approached it, therefore it was estimated that a slightly less amount of serum (perhaps 0.7 c.c.) would have given an equivalent degree of haemolysis. So the point was located accordingly. In the same way the tubes of the remaining series were compared with the "standard" and corresponding values were estimated and plotted in the diagram. Parallel colorimetric curves with the same general contour were also derived by taking as "standard color" tubes those with greater or less degree of haemolysis (color) than the one used in the particular case which was plotted. Several such curves in an experiment act as controls upon each other, and upon the test as a whole. For experiments where more exact colorimetric curves are to be derived, the amounts of the active serum or substances used should be much closer together than in this case, since rather wide gaps exist between the amounts of serum used in the con-

secutive tubes. After considerable experience the readings and estimations can be made with a high degree of accuracy.

In a rough way this is the general method which is in common use in comparative haemolytic work when constructing a colorimetric curve. But in some cases, instead of selecting one of the tubes from

TABLE IV (a)
INDIVIDUAL VARIATION OF BLOOD CELL RESISTANCE TO HAEMOLYSIS

Amount Goat Serum.	Blood Cells from Different Horses.					
	No. 323.	No. 338.	No. 343.	No. 350.	No. 352.	No. 353.
1.00 c.c.	++	+	++	++	++	++
0.80 "	++	+	+	++	+	+
0.50 "	+	+	sl. +	+	sl. +	sl. +
0.30 "	sl. +	sl. +	sl. +	sl. +	t.	t.
0.20 "	t.	sl. +	t.	sl. t.	sl. t.	sl. t.
0.10 "	t.	t.	t.	o	o	o
0.05 "	sl. t.	sl. t.	sl. t.	o	o	o
0.02 "	o	o	sl. t.	o	o	o

DIAGRAM IV (b)
COMPARATIVE RESULTS FROM TABLE IV (a)

the series as a "standard," as in this case, a special colorimetric series is made by "laking" a given volume of red blood cells in distilled water. Then giving that solution a numerical value of 100, further dilutions are made from the original by adding more distilled water so that tubes may be prepared, showing 90 per cent., 85 per cent., etc. This allows relative percentage readings to be made of the haemolysis in a series of tubes in a test, provided the number of blood cells have been sufficiently limited; haemolysis of large quantities of blood corpuscles give a very deep coloration, which is difficult to read unless diluted out. This diagrammatic method will be used in connection with other tables, but must not be confused with certain curves which will appear and be explained later. A variation exists in lytic susceptibility of the different blood cells when acted upon by the same haemolytic serum. It is possible that the serum from another goat might have acted differently if it had been tested in parallel on the same blood cells. But this was not tried at the time.

Plainly this brings out the importance of using only the blood corpuscles of one animal in any particular haemolytic test. That is, the blood of one individual should not be used in one portion of a test embracing a series of haemolytic sera, and the blood of a second individual be used in another portion even if the percentages are equal, since this introduces a serious error in the results. Of course where one animal will not furnish sufficient blood for a test, the blood of a second or third can be drawn, and all well mixed, after washing. Then of the mixture ample suspension can be made up for the entire test just before use. Blood suspensions should always be made from freshly washed corpuscles shortly before adding to the tubes of the prepared haemolytic series, then well shaken at once to insure rapid and uniform mixing.

B. COMPLEMENTING SERA

I—Comparative Complementing Values of Rabbit, Sheep and Goat Sera with a Specific Immune Serum

While it is a well-known fact that all fresh blood serum contains complement, it is not commonly appreciated that the activity of sera from different species varies greatly one from the other in this respect.

The same holds true, to less extent, for sera of individuals of the same species. Some workers apparently hold to the view that the proper complementing serum should be derived from a normal animal either of the same species as the animal which has been immunized (against blood cells) or from the species which furnished the red blood cells for the immunization. For efficiency of complement, serum from either source is often inferior to that taken from other species. The experiment as recorded in Table V very well demonstrates this proposition.

A normal goat had been immunized against blood cells of sheep by repeated subcutaneous injections until its serum became strongly haemolytic to those cells. It may be recalled that normal goat serum contains little or no natural immune body against blood cells of sheep. Three series of test tubes had decreasing amounts of the immune serum added, as indicated in left hand column; the complement naturally present had become inactive by the ageing of the serum. To one

TABLE V
RELATIVE COMPLEMENTING VALUES OF SERUM FROM RABBIT, SHEEP AND GOAT

Immune Serum. (Goat).	Compl. Serum. (Rabbit) 0.20 c.c.	Compl. Serum. (Sheep) 0.20 c.c.	Compl. Serum. (Goat) 0.20 c.c.
0.20 c.c.	+++	sl. +	t.
0.15 "	+++	t.	sl. t.
0.10 "	+++	o	o
0.08 "	++	o	o
0.06 "	++	o	o
0.05 "	++	o	o
0.04 "	++	o	o
0.03 "	+	o	o
0.02 "	+	o	o
0.01 "	sl. +	o	o

series, 0.20 c.c. rabbit serum per tube was added; to the second series, the same amount of sheep serum; and to the third, an equal amount of goat serum. The serum for complement in each instance was taken from normal animals, and used while fresh. The technic employed was the same as in the previous tests; the blood suspension was made from well washed normal sheep blood cells. The results show that there is a

marked difference in the complementing values of the respective sera for this particular immune body.

It was not the serum from the species which supplied the immune body (goat), but the serum from another species (rabbit), which showed the greatest complementing activity. The normal goat and sheep sera were equal in complement content, but far lower than rabbit serum. This shows the importance of a proper selection of normal serum for haemolytic work to be most efficient. Certain other factors also enter into the above experiment, but these will be discussed in connection with the following experiments.

II—Comparative Complementing Values of Guinea Pig Serum and Rabbit Serum with a Specific Immune Serum

In order to compare the sera of animals of the same species to each other, and this whole series to a second series of another species as to complementing values, the following test was undertaken: Three normal guinea pigs and the same number of rabbits were bled to death from carotids (ether anaesthesia), and the serum removed by clotting. Six series of tubes were prepared, as indicated in Table VIa, and to each

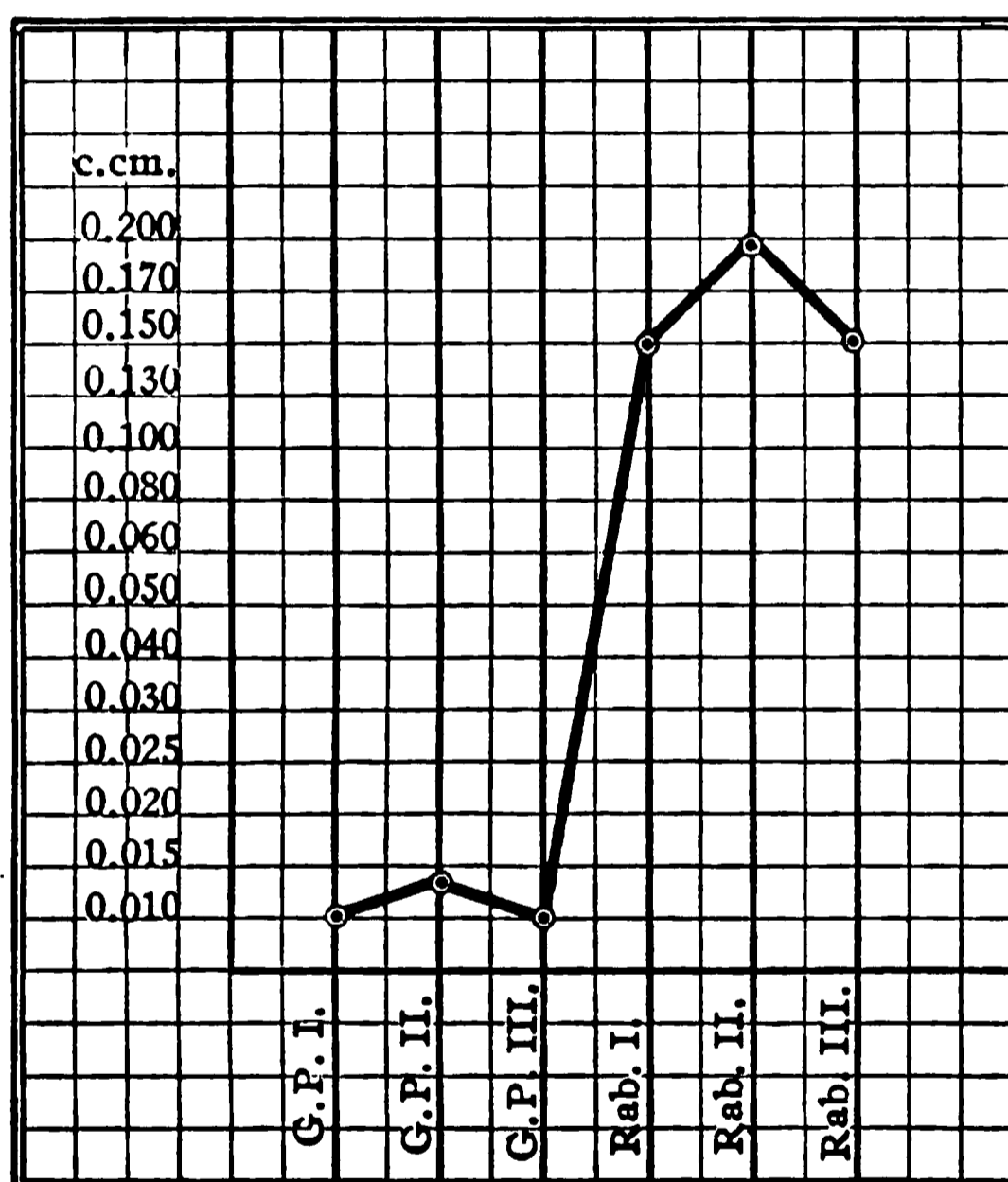
TABLE VI (a)

RELATIVE COMPLEMENTING VALUES OF A SERIES OF GUINEA PIG SERA AS COMPARED TO A SERIES OF RABBIT SERA

Amount of Compl. Serum.	G. P. I.	G. P. II.	G. P. III.	Rab. I.	Rab. II.	Rab. III.
0.20 c.c.	Comp. H.	Comp. H.	Comp. H.	++	+	++
0.17 "	"	"	"	+	sl. +	+
0.15 "	"	"	"	sl. +	sl. t.	sl. +
0.13 "	"	"	"	t.	o	t.
0.10 "	"	"	"	o	o	o
0.08 "	"	"	"	o	o	o
0.06 "	"	"	"	o	o	o
0.05 "	"	"	"	o	o	o
0.04 "	"	+++	"	o	o	o
0.03 "	"	++	+++	o	o	o
0.025 "	++	++	++	o	o	o
0.020 "	++	+	++	o	o	o
0.015 "	+	+	+	o	o	o
0.010 "	sl. +	t.	sl. +	o	o	o

tube was added 0.02 c.c. serum from a goat highly immunized against blood cells of sheep. Then complementing serum from the different animals was added in decreasing amounts, as shown in the column on the left. The series of tubes contain a constant amount of the same immune body, with variable amounts of the respective complement, as indicated at the head of each column. The regular technic was carried out as to blood suspensions, volume, incubation, etc.

DIAGRAM VI (b)
RESULTS OF TEST, FROM TABLE VI (b)



The result may be had by a glance at diagram VIb; it will be noted that in this particular case the series of guinea pig sera contains much more active complement than the series of rabbit sera. Moreover the guinea pig sera were about equal in complement content; the rabbit sera were more variable, one sample in particular was considerably weaker than the other two in complement stuff. Experience shows that for all practical purposes the guinea pig furnishes the most active complementing serum. Unfortunately, owing to the small size of the animal, the amount of serum is quite limited. In tests of large series

of haemolytic sera, rabbit serum stands next in importance, since these animals furnish much more active serum than any of the large animals met with in our experience.

III—Comparative Complementing Values of Guinea Pig Serum and Rabbit Serum when Acting upon Homologous Specific Immune Sera from Alien Species of Animals

The question whether complement is a single, simple homogeneous substance found in fresh serum, or a heterogeneous substance showing a multiplicity of closely similar stuffs more or less specific in character, has long been open to debate. As yet the question has not been definitely decided to the satisfaction of all workers in this field.

Without entering this controversy in any way, we feel that it would

TABLE VII

NORMAL SERUM FROM TWO DIFFERENT SPECIES AS COMPLEMENT TO IMMUNE SERUM FROM A THIRD SPECIES

Amount of Compl. Serum.	G. P. COMPLEMENT.		RABBIT COMPLEMENT.	
	*Immune Serum "A."	†Immune Serum "B."	Immune Serum "A."	Immune Serum "B."
0.50 c.c.	Comp.H.	Comp.H.	Comp.H.	Comp.H.
0.40 "	"	"	"	+++
0.30 "	"	"	"	++
0.25 "	+++	"	"	++
0.20 "	++	"	"	+
0.15 "	++	"	++	+
0.10 "	++	"	+	sl. +
0.08 "	+	"	sl.t.	o
0.06 "	+	"	"	o
0.05 "	+	"	o	o
0.04 "	sl. +	"	o	o
0.03 "	"	"	o	o
0.02 "	"	+++	o	o
0.015 "	o	++	o	o
0.010 "	o	+	o	o

*Immune serum from goat against blood cells of sheep.

†Immune serum from rabbit against blood cells of sheep.

be of interest to submit the following experiment, which might add something to the data on the subject.

A goat which had been immunized against red blood cells of sheep furnished a specific haemolytic serum, which will be indicated by "A." A rabbit was immunized in the same way against sheep blood cells, and gave a specific serum which will be designated by "B." A normal guinea pig and a normal rabbit, each had blood drawn, defibrinated and serum separated by centrifuge. Each serum was used fresh as complement. Four series of tubes were prepared, and to each tube was added 0.05 c.c. immune serum—two series from serum "A" and the other two from serum "B." These series were subdivided into groups, each group having "A" and "B" series of immune serum.

In one group guinea pig serum was added in decreasing amounts to the series for complement, while in the other group rabbit serum was added in the same way in parallel. By consulting Table VII the scheme will be readily understood; the left column indicates the amount of complement used.

The results of the test show that the guinea pig complement was more active, in each case, in conjunction with the immune sera, than the rabbit complement. Also, that under its influence (?) immune serum "B" was more strongly haemolytic than immune serum "A."

TABLE VIII

COMPARISON OF NORMAL RABBIT SERUM TO DISEASED RABBIT SERUM FOR COMPLEMENTING VALUES.

Amount of Compl. Serum.	Normal Rabbit "A."	Normal Rabbit "B."	Normal Rabbit "C."	"Snuffle" Rabbit I.	"Snuffle" Rabbit II.	"Snuffle" Rabbit III.
0.15 c.c.	++	Comp. H.	Comp. H.	Comp. H.	Comp. H.	+++
0.13 "	++	"	"	"	+++	++
0.10 "	+	++	++	++	++	++
0.08 "	+	sl. +	++	++	+	+
0.06 "	t.	o	sl. +	+	sl. t.	+
0.05 "	sl. t.	o	sl. t.	+	o	sl. +

While on the other hand, the rabbit complement influenced (?) the immune body "A" to become more haemolytic than immune body "B," although not to the great extent as was the variation between the two series in the guinea pig complement group. These conflicting results would perhaps be explained by one school of workers as being due to

"multiplicity of complements," in each of the normal sera, and that the specific elective action on their part in the two different immune sera gave the paradoxical results. On the other hand it might be urged that either serum, besides carrying immune bodies and complement, also carries other substances which might react primarily with the haemolytic elements, or among themselves in such a way as to secondarily affect the immune body complement complex. It is quite probable that the immune bodies themselves are not absolutely similar in character or structure. Many factors, still little understood, probably influence the haemolytic reactions. In submitting this test, we wish to point out in particular the importance of using only one specific immune serum and one particular complementing serum throughout any series of investigations involving the physico-chemical relations of these bodies.

IV—A Comparison of Sera from Normal Rabbits and Sera from "Snuffle" Rabbits as to Their Relative Complementing Action

Most serum laboratories have had more or less experience with a commonly known rabbit infection called "snuffles." As a general thing these diseased animals either die or undergo a long illness which makes them worthless for many experimental purposes. It was thought worth while to compare the sera from a series of these diseased animals, showing different degrees of acute infection, with the sera from healthy rabbits for value of the complement. Three normal rabbits were bled from the ear veins, while one "snuffle" rabbit was bled in the same way, and two others from carotids.

In each the serum was separated from the clot after the blood had stood in the ice box over night. Series of tubes were prepared according to the scheme given in Table VIII. To each tube was added 0.035 c.c. of specific immune serum of rabbit immunized against blood cells of sheep. Complementing serum from the different rabbits was added in amounts shown in first columns. Blood suspension, as in all previous tests, was added, and the routine technic was carried out. The result may be seen from the table.

It is quite interesting to note that the average values of the complementing serum from the "snuffles" rabbits was a little higher than that of the normal rabbit. For some purposes these diseased animals

are of equal value for complement as the normal rabbit, and in our experience have been used to furnish complement for testing the haemolytic values of large series of samples of specific immune sera. The results were apparently accurate in every respect, as compared with sera from normal animals. Perhaps the use of such serum would not be so good for tests involving "fixation of complement" or others of the same character. These conclusions are only for this single infection. Other infections have not been tested by us.

C. HAEMOLYSIS AS AFFECTED BY CONCENTRATION OF DIFFERENT COMPONENTS OF THE IMMUNE BODY COMPLEMENT COMPLEX

1—Constant Amount of Complement, Constant Volume of Fluid, Different Concentrations of Blood Cells, and Decreasing Amounts of Immune Body

Certain investigators have carried out work upon the immune body complement complex from the physico-chemical standpoint. It has been shown that haemolytic action apparently follows certain recognized physico-chemical laws, and that the reactions may be expressed by mathematical formulæ. We shall not go into this side further than to call attention to certain combinations which have a practical bearing from the technical standpoint. Such conditions may arise during the course of determining the "immunity curve" in a series of sera derived from an animal immunized against blood cells. In this connection we shall point out the error arising when a perfectly homogeneous blood suspension is not used—that is, when a variable number of blood cells are present in equal volumes of fluid in the same series.

An experiment demonstrating the variations in haemolysis under such conditions was carried out as follows: Five series (reading from top to bottom of table) of test tubes were arranged, and to each series were added decreasing amounts of immune body (serum of rabbit immunized against sheep corpuscles), as indicated in Table IX (a). Each tube then received 0.2 c.c. fresh serum from normal rabbit for complement, and 0.9 per cent. salt solution enough to bring the volume of immune body and complement up to 1.0 c.c. in each tube. Finally different blood suspensions were prepared of such concentration that when 2 c.c. were added to the respective series of tubes, the resulting

mixture (3 c.c. vol. in each tube) gave the percentage as indicated in the table, 1 per cent., 2 per cent., 3 per cent., etc.

The mixtures in the tubes were well shaken and then incubated at 37° C. for one hour, again shaken and placed in the ice box over night. Comparative colorimetric readings of a given degree of haemolysis were reckoned throughout the entire series, and designated by the character Δ .

TABLE IX (a)
HAEMOLYSIS AS INFLUENCED BY BLOOD CELL CONCENTRATION IN CONSTANT VOLUME OF FLUID

Amt. Immune Body	Blood suspension, Vol., 3 c. c.				
	1%	2%	3%	4%	5%
0.005 c.c.	—	—	—	—	Δ
0.004 "	—	—	Δ	Δ	Δ
0.003 "	—	—	Δ	Δ	—
0.0025 "	—	Δ	—	—	—
0.0020 "	—	Δ	—	—	—
0.0015 "	Δ	—	—	—	—
0.0010 "	—	—	—	—	—

By referring to table IX (a), it is evident that, by another arrangement, the test tubes would fall into series different from those just given. That is, reading from left to right on the horizontal line seven series of tubes would be formed. Each of the tubes in any given series (as, for example, the line headed by 0.005 c.c. immune body), would contain the same amount of immune body, the same amount of complementing serum, the same volume of fluid, but different numbers of blood cells (concentrations) as compared to others in the same series. A colorimetric curve could have been constructed on this basis

Note.— Δ "standard color" tube selected from 1% series to make comparison for colorimetric curve.

Δ "standard color" would fall about half way between the two tubes indicated.

Δ "standard color" would fall very close to the tube connected by the three lines below.

by making a direct percentage reading of the amount of "laking" or haemolysis shown in each tube by comparing with a series of standard percentage color tubes, as explained under Section A, Division IV. Such a reading would show a decrease from greater to less haemolysis as we pass from left to right, or from a less to a greater blood cell concentration (1 per cent. to 5 per cent.). But similar results may be deduced from the colorimetric curve IXc (equal degree of haemolysis), which is given in connection with this discussion.

To return to the colorimetric readings shown in the table, we must add that such readings can be controlled in some cases by comparing "observed values" in each series with "estimated values" when the reaction follows a definite law which is expressible by means of a known mathematical formula. In this case the numerical value of Δ in the experiment was determined by taking the amount of immune body indicated, at that point in each series where the haemolysis (color) corresponded with the original "*standard*" tube Δ . The sixth tube from the top in the "1 per cent. series" was used as a "*standard color*" tube. For the construction of a curve representing the results of the test, the reciprocal values were taken of the corresponding amounts of immune body (closely approximated in some cases), as indicated by Δ in each column of the different series.

The table IXb, giving the "observed values" and the "reciprocal

TABLE IX (b)
NUMERICAL VALUES FROM TABLE IX (a) FOR "COLOR CURVE"

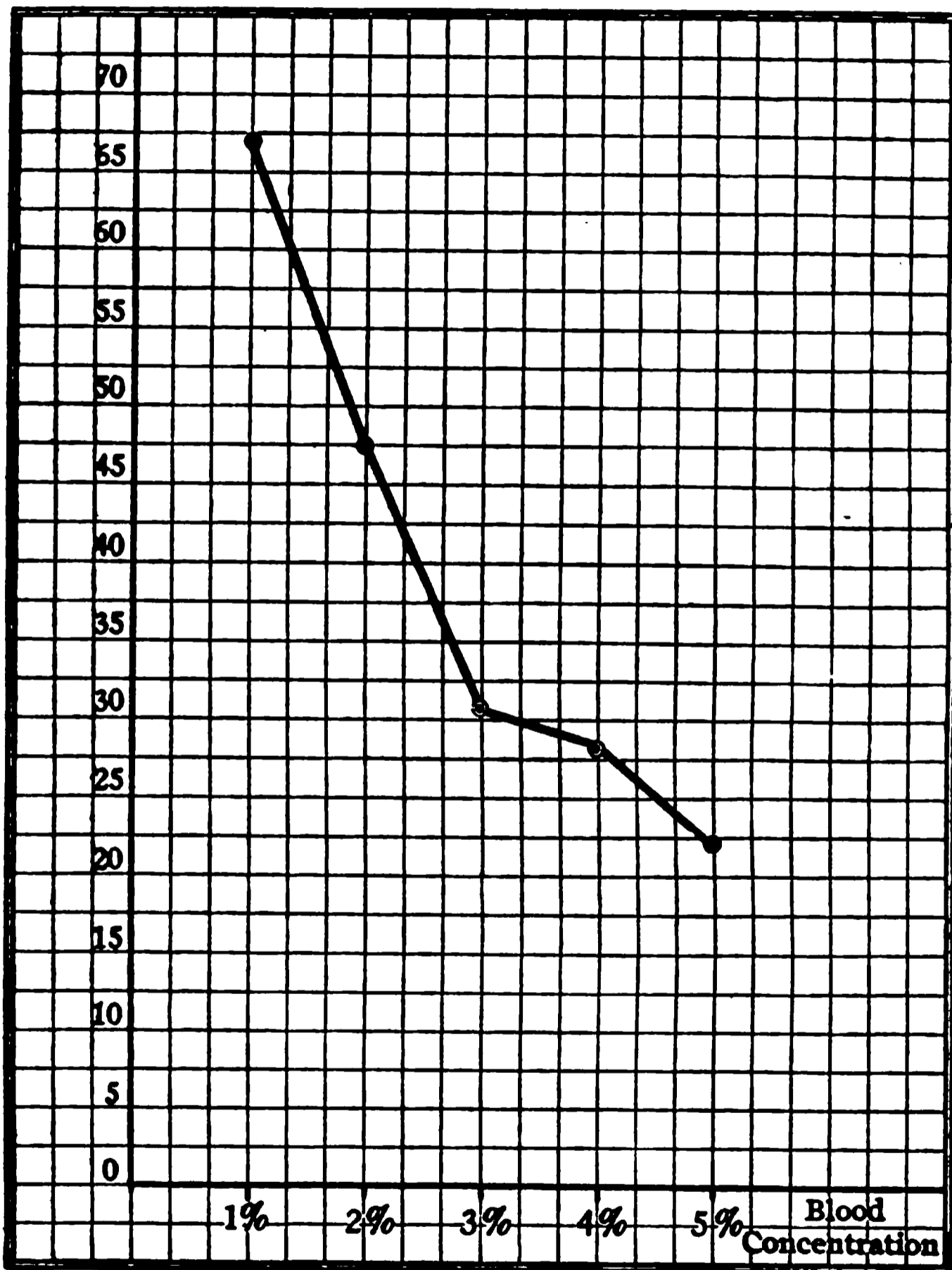
Percentage Series.	Observed Values.	Reciprocal Values.
1%	0.0015	667
2%	0.0021	476
3%	0.0032	313
4%	0.0035	286
5%	0.0045	222

values," will make the method clear. In plotting the curve (curve IXc) only 1-10 of the reciprocal values have been taken, since that scale answers fully as well to bring out the relative values between

the series, the object of the test. The ordinates show the reciprocal values derived (IXb), while the abscissa indicates the different percentage of blood concentrations in the series. In a general way this

CURVE IX (c)

CURVE SHOWING THE INFLUENCE OF BLOOD CONCENTRATION UPON HAEMOLYSIS



experiment is parallel to the test ordinarily used to determine the haemolytic value of a specific immune body in any series of sera. But in this case our serum was used throughout with five different percentages of blood, instead of only one definite percentage of blood cells. The results of this test show that a marked variation in haemo-

lysis does take place when all factors, excepting the number of blood corpuscles present are constant. A higher degree of haemolysis was produced in the lower percentages of blood dilutions, as shown upon plotted curve. Therefore, during any experiment or test of this character it is highly important that the blood mixture (blood cells in 0.9 per cent. NaCl solution) be kept well shaken during the pipetting off of the blood. The upper and lower layers of the blood mixture after 20 to 30 minutes may vary markedly as to concentration of corpuscles. Obviously this may be a means of pronounced error in the final results.

II—Constant Amount of Complement, Variable Volume of Fluid, Constant Number (Approximate) of Blood Cells and Decreasing Amounts of Immune Body

In connection with the previous experiment it was thought to be of interest to vary the concentration of the different elements of the immune body complements complex, with constant number (approximate) of blood cells. Five series of tubes were prepared, reading from top to bottom in Table Xa, and to each of these series were added decreasing amounts of a specific haemolytic serum (rabbit serum against sheep blood cells); each tube had an equal volume of complement, 0.10 c.c. fresh guinea pig serum.

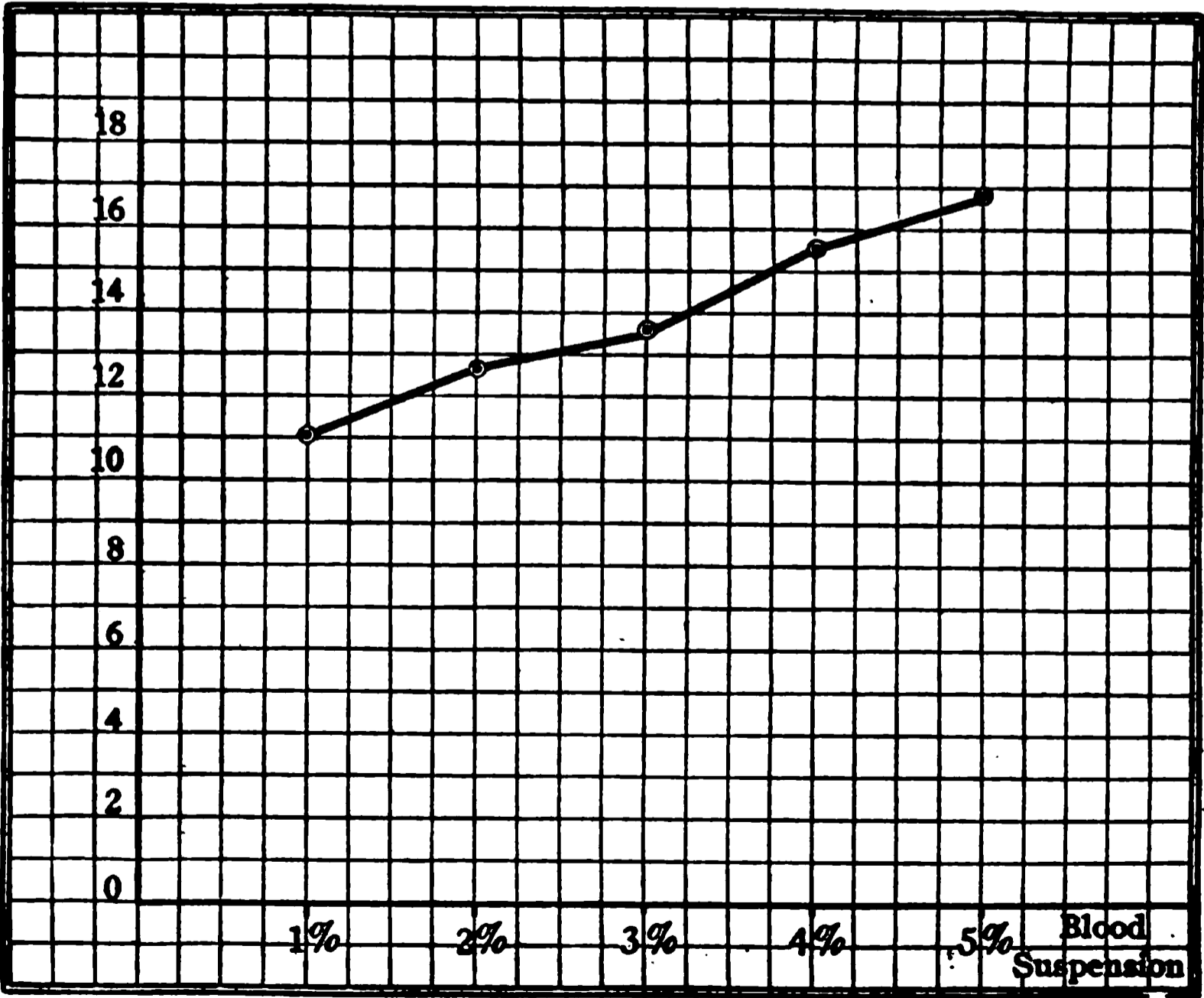
From a concentrated suspension of blood of a definite strength, equal amounts were added to each tube throughout the entire series, giving approximately the same number of blood cells in each tube. But physiological salt solution was first added to each series, so that the first series contained sufficient fluid to give a 1 per cent. blood suspension of corpuscles in the total mixture; the second series received less salt solution, so that the final mixture was equivalent to a 2 per cent. blood suspension; and so on by lesser dilutions giving series of tubes with 3 per cent., 4 per cent. and 5 per cent. blood suspension. As apparent, the volume of fluid in the 1 per cent. series was five times greater than the volume in the 5 per cent. series. And the concentration of the haemolytic elements decreased as the volume of the fluid increased. The technic of the experiment was quite the same as in previous tests. After the reaction between corpuscles, immune body and complement

was completed, sufficient salt solution (0.9 per cent.) was added to the entire series of tubes to make the volume of fluid equal in each throughout. The mixture was well shaken, placed in the ice box to allow the

TABLE X(a)
INFLUENCE OF CONCENTRATION OF IMMUNE BODY, COMPLEMENT, AND BLOOD
SUSPENSION UPON HAEMOLYSIS

Amt. of Immune Serum 1: 200	Constant number of blood cells				
	1%	2%	3%	4%	5%
0.10 c.c.	—	—	—	—	—
0.08 "	—	—	—	—	—
0.06 "	—	—	—	—	—
0.05 "	—	—	—	—	—
0.04 "	—	—	—	—	—

CURVE X (b)
CURVE FROM TABLE X (a)



remaining intact corpuscles to settle out, after which the colorimetric readings were made.

A colorimetric comparison was run through the series, as shown in Table X (a), and from the reciprocal values of the numbers found a curve X (b) was plotted. It will be observed that haemolysis was greater in the series where the mixture of elements was more concentrated (5 per cent.) and diminish downward to the least concentrations (1 per cent.). This experiment might be read in another way by making a special arrangement of the tubes into different type of series. That is, make the reading of the table from left to right along the line which shows, as in the first case, 0.10 c.c. immune serum. In such an arrangement each tube of the series, as shown running from left to right in the table, would contain the same amount of immune body, a constant amount of complementing serum, a constant number of blood cells, but a variable amount of fluid (0.9 per cent. NaCl solution), giving different concentrations of all the elements in the haemolytic system.

This is practically parallel to the table in the section above. A curve could be gotten in the same way as was pointed out in that connection, so we shall refer to that section to save repetition.

When this experiment is compared with the preceding one, a striking contrast is found to exist. In the former case, the concentrations of the haemolytic components were equal in each series—only the number of corpuscles were variable in same volume of fluid. In this case the elements throughout varied in a regular order, as explained. Considered from the standpoint of blood concentrations, it is seen that in comparing the same concentration of both experiments, as the 5 per cent. series, in the first experiment the least haemolysis was induced, while in the second the greatest haemolysis took place. Among the factors which alter the results of haemolytic actions, concentration of the haemolytic elements also plays an important role. As demonstrated in this last test, it is always important to bring the volume of fluid in each test tube to a given volume, constant throughout the series, even when a constant amount of blood and serum is used.

CONCLUDING REMARKS

In submitting the above data, together with the methods used in

developing the same, we trust that certain facts have been brought out which may prove to be of practical value to other workers in this field. While certain parts of the work are only a repetition of work done by other investigators, other materials have been brought forward which may throw light upon particular cases, and serve as a guide to prevent technical errors under certain conditions. Much of the elementary technic has been omitted since that material is available in many of the recent works which cover the subject of immunity.

We wish to acknowledge the assistance rendered by Dr. David Flynn and Miss Helen Hussey in connection with certain parts of the laboratory work.

REPORT OF THE BACTERIOLOGICAL EXAMINATION OF FECES FROM TYPHOID CONVALESCENTS, POST- TYPHOID AND NORMAL CASES FOR THE PRESENCE OF TYPHOID BACILLI

BY

W. CAREY NOBLE and JOSEPHINE S. PRATT

In the early spring of 1907 Dr. Goodwin, of this laboratory, isolated typhoid bacilli from the feces of Mary M., who was suspected by Dr. Soper of being a typhoid carrier. This case has been under our observation ever since, and during this time, the stools tested every week, with few exceptions, have continued to show many typhoid bacilli. (See Vol. III Collected Studies.¹)

The investigation of this typhoid carrier suggested to us first, the study of the persistence of typhoid bacilli in the stools of patients who are recovering from typhoid fever; second, the persistence of typhoid bacilli in the stools of patients who have passed the convalescent state, and may be classed as post-typhoid cases; and third, the study of stools from normal or non-typhoid cases for the possible presence of typhoid bacilli.

TYPHOID CONVALESCENTS AND POST-TYPHOID CASES

We began our investigation by the study of the persistence of typhoid bacilli in the stools of typhoid convalescents. These were obtained from 118 cases from the following institutions: Bellevue, St. Vincent's, Gouverneur, The City Hospital and Work House, Long Island State Hospital and the Smith Infirmary. To the doctors and nurses of these institutions we wish to express our thanks for aid and interest in the work.

The stools were collected in sterile bottles, care being taken that they should be uncontaminated by urine, and were brought to us as soon after collection as possible. A loopful of feces was then diluted in a sterile broth tube, and a loopful from the tube was streaked over a Conradi-Drigalski plate. Usually three or four plates were made from

¹Report on the Bacteriological Examination of a Typhoid Carrier. Collected Studies from the Research Laboratory, 1907, p. 193.

each specimen of feces. These plates, after incubation for 24 hours at 37°C., were examined for typhoid-like colonies. These colonies were fished into sterile nutrient broth and incubated for 24 hours at 37°C. They were then tested for agglutination with a high-grade anti-typhoid horse serum. The hanging drop method was used and a laboratory typhoid culture (Mt. S.) was used as a control. The cultures which agglutinated were then tested on the following media: broth, agar-agar, gelatin-slab, gelatin plate, litmus milk, glucose peptone water fermentation tubes, neutral red lactose peptone water fermentation tubes, potato and Dunham's peptone solution (a test for indol being made after ten days' incubation at 37°C.). The growth on the different media was compared with that of the laboratory culture of typhoid (Mt. S.) used as control.

In planning the work, it was our hope to be able to make at least three examinations from each case, as we realized that a negative result from one stool was by no means indicative of the absence of typhoid bacilli in the feces. Unfortunately, however, we were not able to do this in many cases, for a report of a negative result of an examination was sometimes misinterpreted and a report of a positive result was sometimes disregarded and the patient was discharged from the hospital while still in a condition to spread the disease. In 50 of the 118 cases but one stool was obtained.

Of these 118 convalescent cases of typhoid, 15, or 12.7 per cent., retained the bacilli in the feces after the temperature had become normal. Five cases were discharged from the hospital, while their stools still showed enormous numbers of the bacilli; eight cases retained the bacilli from one to three weeks and two cases have been under observation for one and one-half years and are still positive.

The number of stools examined from the 15 cases in which typhoid bacilli were found is given on following page.

We found here, as in our previous work, that the proportion of typhoid organisms varies greatly. A positive stool showing only one or two typhoid colonies per plate would often be followed by one showing almost a pure culture of typhoid, or a negative stool would be followed by a positive one.

Case Number.	Number of Stools Examined.	Stools in Which Typhoid Bacilli Were Found.
1	12	1
2	9	1
3	6	1
4	4	1
5	4	2
6	7	2
7	24	18
8	34	6
9	3	1
10	1	1
11	1	1
12	1	1
13	1	1
14	65	53
15	67	54
Total 15 cases	239 Stools Examined	144 Positive Stools

As may be seen below, most of those who still harbored typhoid bacilli in their feces during convalescence did so for about one to three weeks only. Of the five who were discharged from the hospital while their stools still showed enormous numbers of typhoid bacilli we were able to trace only one.

Two tests of this patient's feces made three months later were negative for typhoid. Cases 14 and 15 have been under observation for one and one-half years and are still showing large numbers of typhoid in the stools.

	Sex.	Temperature Normal.	Result.	Remarks.
Case 1	F.	1st Test, 5 Days 2nd " 7 " 3rd " 8 " 4th " 9 " 5th " 11 " 6th " 12 " 7th " 14 " 8th " 22 " 9th " 23 " 10th " 25 " 11th " 28 " 12th " 35 "	— + — — — — — — — — —	Case was discharged from the Hospital.
Case 2	F.	1st Test, 4 Days 2nd " 9 " 3rd " 10 " 4th " 11 " 5th " 12 " 6th " 13 " 7th " 16 " 8th " 17 " 9th " 26 "	+ — — — — — — — —	Case was discharged from the Hospital.
Case 3	F.	1st Test, 7 Days 2nd " 17 " 3rd " 26 " 4th " 41 " 5th " 47 " 6th " 54 "	+ — — — — —	Patient discharged from the Hospital.
Case 4	M.	1st Test, 7 Days 2nd " 27 " 3rd " 34 " 4th " 41 "	+ — — —	Patient discharged from the Hospital.
Case 5	M.	1st Test, 15 Days 2nd " 16 " 3rd " 28 " 4th " 32 "	+ + — —	Patient discharged from the Hospital.
Case 6	F.	1st Test, 17 Days 2nd " 19 " Relapse 3rd Test, 10 Days 4th " 17 " 5th " 24 " 6th " 31 " 7th " 38 "	— + + — — — —	Patient (an insane woman) had a severe case of typhoid with relapse.

	Sex.	Temperature Normal.	Result.	Remarks.
Case 7	M.	1st Test Dec. 9, '07	+	Patient was still running a temperature when the first 15 tests were made. He was admitted to the Hospital Dec. 8, 1907.
		" 10	+	
		" 11	—	
		Dec. 12, 12, 13, 14, 15	+++++	
		" 16	—	
		" 17, 18, 20, 20	++++	
		" 21, 22	++	
		Dec. 23 T.N. 1 Day	—	
		" 24 2 Days	—	
		" 25 3 "	+	
		Jan. 1, '08 7 "	+	Patient was discharged from the Hospital.
		14 "	+	
		16 "	+	
		17 "	+	
		20 "	—	
		21 "	—	
Case 8	M.	Temp. not yet normal		Case was discharged from the Hospital.
		1st Test	+	
		2nd "	—	
		2nd & 4th Tests	++	
		5-6-7-8-9-10-11-12-13-14 (1st 14 tests were made on successive days.)	—(10x)	
		5 days later, Nov. 13	+	
		Nov. 14, 15, 16, 18	—(4x)	
		Nov. 19 T.N. 1 Day	+	
		" 20 " 2 Days	—	
		" 21 3 "	+	
		" 22, 23	---	
		" 25, 26	---	
		" 27, 29, 30	-----	
		Dec. 3, 4, 5, 6, 7	-----	
		" 27, 30	---	
Case 9	M.	T.N., 12 Days	+	Almost pure culture of typhoid bacilli. Case was discharged from Hospital a day or two later, before a second test was made.
		2nd Test—3 Months later	—	
		3rd " —3 " & 7 Days	—	

	Sex.	Temperature Normal.	Result.	Remarks.
Case 10	M.	1st Test—3 Days	+ (80 % of colonies were typhoid.)	Case was discharged from Hospital before a second test was made.
Case 11	M.	1st Test—21 Days	+	Two days later, case was discharged from the Hospital and no tests could be made.
Case 12	M.	1st Test—7 Days	+	Case was discharged from the Hospital without further tests.
Case 13	M.	1st Test—14 Days	+	Case was discharged from the Hospital without further tests.

The next two cases are given in full as follows:

Case 14. An insane woman who had a light case of typhoid in October, 1907:

	Result.
Nov. 6—1st examination of feces made when temperature normal 7 days.....	+
" 13.....	—
" 20, 27.....	++
Dec. 4.....	—
" 11, 18, 27.....	+++
<hr/>	
1908	
Jan. 2, 8, 15, 22, 29.....	+++++
Feb. 5, 12, 19, 26.....	++++
Mar. 4, 11, 18.....	+++
" 25.....	—
Apr. 8, 15.....	++
" 29.....	—
May 6, 13, 20, 27.....	++++
June 3, 10, 17, 24.....	++++
July 1.....	—
" 6, 15, 22, 29—Aug. 9.....	+++++
Aug. 12.....	—
" 19.....	+
" 27.....	—
Sept. 2, 9, 17.....	+++
" 24.....	—
" 30—Oct. 7, 14.....	+++
Oct. 22.....	—
" 28—Nov. 4, 11, 25.....	++++
Dec. 8, 10, 24.....	+++
" 29.....	—
<hr/>	
1909	
Jan. 6, 13, 26.....	+++
<hr/>	
Total.....	60 examinations, 50 stools in which typhoid bacilli were found.

Case 15. An insane woman who had a light case of typhoid in September, 1907:

	Result.
1st stool tested Oct. 23, temperature normal	
14 days.....	—
Oct. 31.....	+
Nov. 6.....	—
" 13, 20, 27.....	+++
Dec. 4.....	—
" 11, 18.....	++
" 27 & Jan. 1, 1908.....	—
—	— —
1908	+++
Jan. 8, 15, 22.....	++++
" 29.....	++++
Feb. 5, 12, 19, 26.....	++++
Mar. 4, 11, 18, 25.....	— —
Apr. 8, 15, 29–May 6.....	+++++
May 3, 20.....	++
" 27–June 3, 10, 17, 24.....	—
July 1, 6.....	++++
" 15.....	++++
" 22, 29–Aug. 5, 12.....	—
Aug. 19, 27–Sept. 2, 9.....	++++
Sept. 17.....	—
" 24, 30–Oct. 7, 14.....	++++
Oct. 22.....	++
" 28–Nov. 4, 11, 25.....	— —
Dec. 8, 10.....	
" 24, 29.....	
—	+++
1909	
Jan. 6, 13, 26.....	
Total number of stools tested, 62, of which 49 were found to contain typhoid bacilli.	

Typhoid found = +
Typhoid not found = —

The second set of cases which we have classed as post-typhoid was obtained from the New Jersey State Hospital at Trenton. During the summer and early fall, 1907, there had been an outbreak of typhoid in the institution. Five months later an examination was made of the stools of the inmates who had had typhoid at that time. There were 54 cases which were examined as follows:

8 cases.....	1 stool examined
31 "	2 stools "
13 "	3 " "
1 case	4 " "
1 "	5 " "
<hr/> Total 54	<hr/> 118

Two of these cases were found still harboring typhoid bacilli: cases 16 and 17. Case 16 died in October, 1908, but not from typhoid. Case 17 is still under observation.

		Result
Case 16	Stool tested 5 mos. after typhoid	+
	" " 6 " " "	+
	" " 7 " " "	+
	" " 8 " " "	+
Case 17	Stool tested 5 mos. after typhoid	+
	" " 6 " " "	—
	" " 7 " " "	—
	" " 8 " " "	—
	" " 1 yr. 10 mos. after typhoid	+

Cultures from the 17 cases in which stools were found to contain typhoid bacilli were tested for agglutination with an anti-typhoid horse serum as shown in the following tables. A culture of *B. coli* was also tested, as well as a laboratory typhoid culture Mt. S., which was used as control.

TABLE I
ANTI-TYPHOID HORSE SERUM

	Control of Broth Suspension.	1-2000	1-5000	1-10,000	1-20,000	1-40,000
B. Coli	—	—	—	—	—	—
Mt. S.	—	++	+I	+	+	± (—)
Isolation 1	—	+	+	±	± (—)	I
" 2	—	+	+	±	—	—
" 3	—	+	±	± (—)	I	—
" 4	—	+	+	±	I	—
" 5	—	+I	+	+	±	± (—)
" 6	—	++	++	+I	+	±
" 7	—	+	+	±	±	I
" 8	—	+	+	±	± (—)	I
" 9	—	+	+	±	±	I
" 10	—	+	±	±	± (—)	± (—)
" 11	—	+	±	± (—)	I	I
" 12	—	+	±	± (—)	—	—
" 13	—	+	±	±	+	I
" 14	—	++	+I	+	+	±
" 15	—	++	++	+	+	± (—)
" 16	—	+	+	+	+	±
" 17	—	++	++	+I	+	±

++ = Absolute agglutination.
 +I = Almost complete agglutination.
 + = Good agglutination.
 ± = Fair agglutination.
 I = Tendency.
 — = No agglutination.

Each culture was also added to the anti-typhoid horse serum in sufficient amount to absorb the greater part of the agglutins. In every case the specific typhoid agglutinin was largely absorbed, as much so as by our laboratory culture. The results with the last seven cultures are given below. The others were very similar.

Absorption of agglutinins in anti-typhoid horse serum by Isolation 11

Dilution of Filtrate.	1-20	1-40	1-80	1-160	1-320	1-640	1-1280	C.
Mt.S.....	++	++	+I	+I	+	±	—	—
Isolation 11.....	+I	+I	+	±	I	I	—	—

Absorption of agglutinins in anti-typhoid horse serum by Isolation 12

Dilution of Filtrate.	1-20	1-40	1-80	1-160	1-320	1-640	1-1280	C.
Mt.S.....	++	++	+I	+	±	±	I	—
Isolation 12.....	+I	+I	±	I	—	—	—	—

Absorption of agglutinins in anti-typhoid horse serum by Isolation 13

Dilution of Filtrate.	1-20	1-40	1-80	1-160	1-320	1-640	1-1280	1-2560	C.
Mt.S.....	++	++	++	+I	+I	+	±	—	—
Isolation 13.	+	±	I	I	—	—	—	—	—

Absorption of agglutinins in anti-typhoid horse serum by Isolation 14

Dilution of Filtrate.	1-10	1-20	1-40	1-80	1-160	1-320	1-640	1-1280	C.
Mt.S.....	+	±	±	I	I	—	—	—	—
Isolation 14.	+	±	I	—	—	—	—	—	—

Absorption of agglutinins in anti-typhoid horse serum by Isolation 15

Dilution of Filtrate.	1-20	1-40	1-80	1-160	1-320	1-640	1-1280	C.
Mt.S.....	++	++	++	+I	+	I	—	—
Isolation 15.....	++	++	++	+I	+	±	—	—

Absorption of agglutinins in anti-typhoid horse serum by Isolation 16

Dilution of Filtrate.	1-20	1-40	1-80	1-160	1-320	1-640	1-1280	1-2560	C.
Mt.S.....	++	++	+	±	I	—	—	—	—
Isolation 16.	++	+	±	I	I	—	—	—	—

Absorption of agglutinins in anti-typhoid horse serum by Isolation 17

Dilution of Filtrate.	1-20	1-40	1-80	1-160	1-320	1-640	1-1280	1-2560	C.
Mt.S.....	++	++	++	+I	+	±	I	—	—
Isolation 17.	++	++	++	+I	+	—	—	—	—

The case of the typhoid carrier, Mary M——, which we have mentioned above, has been under observation since March, 1907. The bacteriological examinations of the feces for the first ten months have already been reported; the results of those made in 1908 are as follows:

MARY M.—YEAR 1908

January 2, 8, 15.....	Typhoid present.
January 22, 27.....	Typhoid present.
February 5	Almost pure culture typh.
February 12	25% typhoid colonies.
February 19	7-8 typhoid colonies per plate.
February 26, March 4, 11, 18, 25 }	"few" colonies {
April 1, 9, 15, 29.....	
May 6	Typhoid present.
May 20	No typhoid found.
May 27, June 3, 10, 17, 24, July 1.....	Typhoid present.
July 6, 15	No typhoid found.
July 22	Typhoid present.
July 29	No typhoid found.
August 5, 19, 27.....	Typhoid present.
September 2, 7	Typhoid present.
September 17	No typhoid found.
September 24 ..	Typhoid present.
September 30	No typhoid found.
October 7	20% typhoid.
October 14, 22, 28.....	Typhoid present.
November 4, 11.....	4-5 typhoid colonies per plate.
November 18, 25.....	10% typhoid colonies.
December 2	30% typhoid colonies.
December 10	40% typhoid colonies.
December 7	2-3 colonies typh. per plate.
December 17	6-7 typhoid per plate.
December 23	3-4 typhoid per plate.
December 30	3-4 typhoid per plate.

NORMAL CASES HAVING NO TYPHOID HISTORY

Of the normal cases examined, there were 188, one stool being tested in each case. No typhoid bacilli were found.

REPORT OF BACTERIOLOGICAL EXAMINATION OF CROTON TAP WATER FOR THE YEAR 1908

By W. CAREY NOBLE

Croton tap water at East 16th Street was plated in agar and tested for the presence of colon bacilli once a week during the year. The colony count at 37° C. and 24° C. and the result of the presumptive test were as follows:

	1 c.c. Plated in Agar 37° C. for 24 hours.	At 24° C. 72 hours.	Quantity of Water Con- taining Colon Bacilli as Shown by the Pre- sumptive Test (Lac- tose peptone water).
Jan. 3, 1908	24 colonies	122 colonies	1 c. c.
" 7	27 "	163 "	10 "
" 15	84 "	159 "	1 "
" 21	73 "	218 "	1 "
" 30	18 "	34 "	10 "
Feb. 4	42 "	137 "	10 "
" 13	37 "	84 "	10 "
" 19	52 "	136 "	9 "
" 25	106 "	127 "	1 "
Mar. 3	34 "	306 "	1 "
" 10	15 "	162 "	1 "
" 17	20 "	63 "	10 "
" 24	18 "	46 "	10 "
" 31	27 "	132 "	0.1 "
Apr. 6	22 "	115 "	10 "
" 15	144 "	1370 "	0.1 "
" 21	60 "	321 "	0.1 "
" 28	90 "	167 "	0.1 "
May 5	32 "	134 "	1 "
" 12	67 "	135 "	1 "
" 19	62 "	70 "	0.1 "
" 26	59 "	920 "	0.1 "
June 3	30 "	96 "	0.1 "
" 9	31 "	97 "	0.1 "
" 17	82 "	152 "	0.1 "
" 24	28 "	124 "	1 "

	1 c.c. Plated in Agar 37° C. for 24 hours.	At 24° C. 72 hours.	Quantity of Water Con- taining Colon Bacilli as Shown by the Pre- sumptive Test (Lac- tose peptone water).
July 1	48 colonies	276 colonies	1 c. c.
" 8	40 "	90 "	0.1 "
" 15	75 "	337 "	0.1 "
" 22	59 "	320 "	0.1 "
" 29	139 "	432 "	0.1 "
Aug. 5	63 "	171 "	0.1 "
" 12	1289 "	3160 "	0.01 "
" 20	137 "	268 "	0.01 "
" 26	178 "	316 "	0.01 "
Sept. 2	217 "	640 "	0.01 "
" 9	368 "	283 "	0.01 "
" 16	130 "	177 "	0.01 "
" 24	106 "	238 "	0.1 "
" 30	82 "	139 "	0.1 "
Oct. 8	102 "	201 "	0.1 "
" 15	327 "	710 "	0.01 "
" 20	115 "	211 "	0.1 "
" 29	50 "	190 "	0.1 "
Nov. 4	28 "	132 "	0.1 "
" 12	97 "	228 "	1 "
" 18	44 "	121 "	0.2 "
" 25	90 "	186 "	0.02 "
Dec. 1	25 "	60 "	0.1 "
" 10	38 "	77 "	0.05 "
" 16	10 "	46 "	1 "
" 24	90 "	156 "	10 "
tank on roof			
Dec. 31	1030 "	1090 "	0.02 "

THE QUANTITATIVE CHANGES IN THE PROTEINS IN THE BLOOD PLASMA OF HORSES IN THE COURSE OF IMMUNIZATION

BY

EDWIN J. BANZHAF AND ROBERT B. GIBSON

When an animal is immunized against bacterial toxins (Seng,¹ Joachim,² Atkinson,³ Ledingham⁴) and against foreign proteins (Moll⁵), there result certain characteristic quantitative changes in the proteins of the blood plasma. The content of serumglobulin is strikingly increased, even up to double the normal amount; at the same time there is a diminution in the serumalbumin. Similar changes have been said to occur during starvation,⁶ Moll, however, has denied that the inanition is the cause of the globulin increase observed in precipitin sera.

Atkinson⁷ showed that this serumglobulin increase is in some degree proportional to the antitoxic (diphtheria) potency of the serum. His analyses give figures for the protein precipitable on saturation with magnesium sulphate; this "globulin"⁸ paralleled more or less the gross changes in the antitoxic potency in the serum for the one horse so examined. With his other observations on the increased "globulin" and the antitoxic content of antitoxic serum, a more than incidental relationship between the serumglobulin increase and the antitoxic content is suggested.

In a paper published while the present investigation was under way, Ledingham⁹ gives results of the determination of the total protein, the serumglobulin and the serumalbumin in the course of immunization against diphtheria toxin. The serum was obtained at short intervals from two horses and a goat. With one horse, which failed to yield a high grade antitoxin, the serumglobulin content of the serum was not essentially increased over the normal. The other horse gave ultimately a 650 unit serum; the serumglobulin, here, had progressively increased along with the antitoxic potency. A goat serum which had reached 40 units showed a rise in both the serumalbumin and (to a much less degree) of the serumglobulin over the normal figures. From

Ledingham's results on the two horses, a relation between the development of the antitoxic properties and the increase in the serumglobulin content is indicated.

The observations of Atkinson and of Ledingham, so far as we are aware, are the only determinations of the quantitative relation of the serumglobulin content and antitoxic potency throughout the course of immunization. The subject is of extreme importance because of the constant association of the anti-substance with the serumglobulin.¹⁰ The serumglobulin increase in this protein during immunization may actually represent the accumulation of protective substances; at least if not identical, the antitoxin may be in some loose chemical combination with the protein. Mellanby¹¹ recently states that even the diphtheria antitoxin is actually a part of the serumglobulin (albumin α).

The poverty of the data as to the blood changes during immunization make additional and more conclusive experiments desirable when the significance of the problem as to the chemical nature of the protective substances is considered. In the present paper we present the results of a large number of analyses upon the protein changes in the plasma of 11 horses subjected to immunization.

We have followed quantitatively the protein changes in the sodium oxalate plasma in a series of 11 horses. At first these were undergoing simultaneous immunization, under the direction of Dr. Wm. H. Park, to both tetanus and diphtheria toxins; the horses were subsequently kept on the toxin to which they responded best individually as regards antitoxin production.¹² The first few injections were made with toxin after the administration of antitoxin, as is the custom at the Research Laboratory. The immunization was forced, i. e., the toxins were administered in relatively large doses, usually in weekly injections. The horses were bled for serum production at about 8, 10 or 14 day intervals, when a maximum antitoxic value was attained as indicated by earlier test bleedings of about 500 c.c. of blood. The regular bleedings for serum production (six to ten liters of blood) were instituted, accordingly, only *after* maximum antitoxic (and protein) changes had occurred, as shown in the tables. The variations during the period of increasing antitoxic potency, therefore, have not been influenced by severe hemorrhage.

Suitability of Individual Horses for Antitoxin Production

As has long been known, certain young and healthy horses seem refractory for the production of diphtheria or tetanus antitoxins; again, certain horses will yield a much more potent antitoxin than others treated in an exactly identical manner. This idiosyncrasy is strikingly brought out in the behavior of the animals used in the present experiments. Thus six (319, 322, 323, 326, 327 and 328) of the eleven horses reacted to diphtheria but not to tetanus toxin; the plasma obtained from these six, at maximum potency, was 550 units per c.c. or above, except in the case of 327, where the strength was 400 units. One horse (320) gave only a potent serum against tetanus while being simultaneously refractory for the production of diphtheria antitoxin. Two horses (321 and 324) failed to react vigorously on immunization with either toxin; another (325) responded in some degree to both. Horse 318 was immunized to a high degree (600 units) to diphtheria toxin; transferred to tetanus, it yielded a very potent serum (300 units), and when returned to the diphtheria service nearly the original potency was re-established. There is then an individual adaptation on the part of the horse for the production of certain antitoxins; for other toxins the horse may be almost completely refractory.

MAXIMUM ANTITOXIN POTENCIES IN UNITS

Horse.	Dip.	Tet.
318	600	300
319	600	4
320	50	90
321	150	2
322	625	5
323	850	5
324	125	1
325	300	25
326	600	1
327	400	1
328	550	1

Note.—Maximum potencies for diphtheria and tetanus antitoxins are, of course, not always here coincident.

Protein determinations were made in duplicate by heat coagulation and weighing on the oxalate plasma, as follows:

1. The total protein.
2. The total protein less the fibrinogen.

The protein in an aliquot of the filtrate from the fibrinogen, precipitated at 2.9 saturation¹³ ammonium sulphate and at an ultimate dilution (when precipitated) of tenfold the original volume of plasma taken for the fractioning.

3. The serumalbumins.

The protein in an aliquot of the filtrate at 5.0 (half saturation) ammonium sulphate at a dilution of the plasma, when precipitated, of tenfold the original volume of plasma taken.

4. (a) The serumglobulins soluble in saturated NaCl solution.

Separated by direct solution in saturated NaCl solution of the proteins precipitated at half saturation ammonium sulphate at an ultimate plasma dilution of 1:10.

(b) Or the protein in solution on saturating the plasma with NaCl.

The several times diluted plasma was saturated with the dry salt and made up to ten times the volume of plasma taken with saturated NaCl solution. Determinations were made on aliquots of the filtrates. This separation was more commonly used than the preceding.

5. The "pseudoglobulin" plus the serumalbumin.

The protein of aliquots of the filtrate from the plasma precipitated at third (3.3) saturation ammonium sulphate and at ultimate dilutions of 1.5, 5 and 10 times the volume of the plasma used.

The volume of the precipitates obtained in the above determinations was so small that but little error should be obtained in employing aliquots of the filtrates as above for the analyses. In the precipitation of the "euglobulin" at 1:1.5 dilution, however, such error must influence the results somewhat; even here we were never dealing with a precipitate from 10 c.c. of oxalate plasma of over 0.5 gm. in a volume, when precipitated, of 15 c.c. Inasmuch as the density of proteins is greater than water, and the volume of solvent is increased somewhat by their solution, the error in the analytical results must certainly be less than 3%, an error perfectly negligible in the interpretation of the figures for the "euglobulin" at the dilution of the plasma of 1:1.5.

From the analytical data so obtained, the content of the plasma in the following protein constituents is available.

The total protein.

Fibrinogen.

Serumalbumins.¹⁴

Serumglobulins.¹⁴

Serumglobulin, insoluble in saturated NaCl solution.

Serumglobulin, soluble in saturated NaCl solution.

"Euglobulin" precipitated at ultimate dilutions of the plasma of 1:1.5, 1:5 and 1:10.

"Pseudoglobulin," similarly precipitated.

Because of the large amount of analytical data obtained, it does not seem advisable to go into details of the variations in each of the 11 horses examined. Certain interesting and important *general* features of the results will be discussed in full and example given from the material at hand. The conduct of the experiment and the results of the analyses are more fully given in the tables at the end of this article. Some graphic representations of the protein and antitoxic changes are also reproduced.

The characteristic serumglobulin increase in the plasma during im-

munization was observed in all the eleven horses. Certain characters of this increase may be discussed.

The maximum increase in the serumglobulin was usually coincident with the maximum antitoxic potency of the plasma. Two exceptions were noted (horses 320 and 324). The maxima were attained in the seventh to ninth week of immunization, but both antitoxin and serumglobulin may be diminished even at the time of the first antitoxin bleeding¹⁸ (319, 322, 323, 325).

The curves of the serumglobulin increase and the development of the antitoxic potency were not otherwise parallel. The maximum serumglobulin content in two horses (320 and 324) was actually reached previous to the highest antitoxic potency.

After immunization for a period of 39 days, horse 320 gave a plasma of 20 antidiphtheria units per c.c., the serumglobulin content of which was increased 42.9% over the normal. The plasma obtained on the 59th day tested up 35 antidiphtheria and 2 antitetanus units; the serumglobulin had increased to its maximum of 87.6% over the normal. Twenty-eight days later the potency had risen to 50 units for each antitoxin, while the serumglobulin increase had fallen to 69.7%. Subsequently, with a further rise in the tetanus antitoxin to 90 units, the serumglobulin had diminished to 48.4% over the normal. For the second horse, 324, antitoxic potencies of 25,125 and 125 units were accompanied by a serumglobulin content above the normal of 59.3%, 48.5% and 38.9% respectively.

With the exception of horses 318 and 319, on which we have no "intermediate" analyses, the other animals all showed a marked increase in the serumglobulin content, *preceding* the development of the antitoxic properties.

For example, 32 days after the combined immunization was started, horse 322 yielded a plasma which tested 50 units of diphtheria antitoxin per c.c. and in which the serumglobulin had been increased 44.8%; 35 days later the plasma tested 625 units and the serumglobulin increase was 90.6%. The antitoxic potency, therefore, had been brought up over 12 times while the serumglobulin increase had been only double during the same period. Similarly, the serumglobulin in the plasma of horse 325 had increased 40.7% after 27 days immunization, the potency being just 5 units; on the 54th day the serumglobulin increase was 90.2%, while the antitoxic potency had risen to 600 units of diphtheria and 1 unit of tetanus antitoxin.

While the greatest rise in the serumglobulin was usually coincident with maximum antitoxic potency, as already pointed out, the extent of this increase in the serumglobulin was practically independent of the antitoxic potency when the results on more than one horse were contrasted. There may be, then, no relation between the absolute or percentage increase of the serumglobulin and the antitoxic potency in the plasma of different horses. The increase in the serumglobulin of re-

fractory horses may surpass that in the plasma of some of those yielding a high antitoxin. The greatest absolute increase in serumglobulin (4.40 gms.), however, was observed in the plasma of the horse (323) which supplied the strongest antitoxin serum of the series. In contrast, the refractory horse 321 showed an absolute increase in the serumglobulin of 4.07 gms. per 100 c.c. plasma.

For horse 318 this increase was 41.1% (2.01 gms. per 100 c.c. plasma); for 319, treated in exactly the same manner during immunization and yielding a plasma of the same potency (600 units diphtheria), the increase was 86.3% (3.66 gms.). Horse 321 showed an increase of 87.6% (4.07 gms.) in the serumglobulin when supplying a plasma of maximum potency of only 150 units per c.c.; this horse failed to react well to the toxin not only as regards antitoxin production, but died subsequently of toxemia when the immunization was continued. Horse 323 gave a maximum of 850 units (diphtheria) and the serumglobulin had been increased 95.6% with the greatest absolute increase (4.40 gms.) we have observed. Horse 328 yielded a 550 unit plasma, the serumglobulin increase being 113.9% (4.00 gms.). The refractory horse 324 is especially interesting. At the third bleeding (5/31/07) a potency of 125 units (diphtheria) and a serumglobulin increase of 48.5% (2.69 gms.) were found; 19 days later (6/19/07) the antitoxic strength was unchanged, but the serumglobulin content had fallen until the increase was only 38.9% above the normal. A similar change was observed for horse 326, where the serumglobulin maximum fell from 7.61 gms. (4/30/07) to 5.92 gms. without change of antitoxic strength; the results here, however, are complicated by the "full" bleeding.

SERUMGLOBULIN INCREASE AT MAXIMUM ANTITOXIC POTENCY

Horse.	Potency.		Serumglobulin.	Increase.
	Dip.	Tet.	Gms per 100 c.c. Plasma.	Per cent.
318	600	1	2.01	41.1
319	600	1	3.66	86.3
320	50	90	2.19	46.2*
321	150**	2	4.07	87.6
322	625	5	3.56	90.6
323	850	5	4.40	95.6
324	125**	1(?)	2.69	48.5*
325	300	4	3.22	89.1
326	600	1	3.61	90.2
327	400	1	2.87	102.1
328	550	1	4.00	113.9

* Maximum serumglobulin increase preceded maximum antitoxic potency.

** Refractory horse.

When the serumglobulin had reached a maximum with maximum antitoxic strength, the protein diminished with the decrease in potency even before regular bleedings were established. It was the practice in the Research Laboratory to bleed the horses for antitoxin production

when test bleedings indicated a high or maximum potency. In some instances the horses reached a maximum, and the antitoxic potency had fallen somewhat by the time the first regular "full" bleeding was instituted. This diminution in potency was accompanied by a lowering of the serumglobulin from the maximum content. This fall, however, seems to have had no quantitative relation to the degree of the potency changes, which in some instances were very slight. Accordingly the lowering of the serumglobulin is probably identical with the submaximal fall in serumglobulin coincident with the highest potency in the plasma of horses 320 and 324, and is characteristic in itself and more or less independent of the antitoxin variations.

For instance, horse 319 gave a maximum of 600 units diphtheria antitoxin and 1 unit of tetanus. The serumglobulin increase was then 3.66 gms. per 100 c.c. of plasma. With a change in antitoxic potency to 550 units of diphtheria and 2 of tetanus antitoxin (bleeding 4/2/07), the serumglobulin increase had fallen to 2.81 gms. Thus for a decrease in the antitoxin properties of about 1-12, the serumglobulin increase had been reduced $\frac{1}{4}$. A fall in the potency of the plasma of horse 322 from 625 units of diphtheria antitoxin and 5 of tetanus, to 500 units of diphtheria (5/3/07) cut down the increase in the serumglobulin from 3.56 to 3.01 gms. Here, then, a fall of 1-24 in potency caused a drop in the increase of serumglobulin of a trifle less than 1-6. Similarly when the plasma of horse 323 fell from a maximum of 850 units of diphtheria and 5 of tetanus antitoxin (4/29/07) to 550 units of diphtheria (5/10/07), the drop of over 35% in the potency was accompanied by a curtailment of 22.5% in the increase of the serumglobulin over the normal.

We have already shown that during immunization the serumglobulin increase tends to precede the development of the antitoxic potency; that the two reach a maximum, usually coincident, and that with the fall in potency, or even before, the increase in the serumglobulin is diminished to greater degree than the potency change. This last decline continued for one-third "full" bleedings, apparently irrespective as to whether the antitoxic properties were maintained or not. Subsequently there was a slight increase in the serumglobulin.

For example, at the first "full" bleeding (4/30/07) of horse 326, the 8 liters of plasma obtained tested 600 units per c.c., and the content in serumglobulin 7.61 gms. per 100 c.c. On the next "full" bleeding (5/10/07) the serumglobulin content of the plasma, of the same potency, had fallen to 5.92 gms. We are not dealing here with the effects of dilution, through bleeding, because the serumalbumin has been increased from 1.16 to 1.44 gms. per 100 c.c. plasma. Eight days later (5/18/07) the potency was still 600 units; the serumglobulin content had increased from 5.92 gms. to 6.96 gms. per 100 c.c. of plasma, while the serumalbumen had fallen from 1.4 gms. to 0.98 gms. Practically the same fall is shown by the analyses in the plasma of horses 319, 320, 323 and 325. For horse 318 similar variations occur, but because of the double immunity here it is impossible to give a comparative value to the potency variations. The latter rise is well brought out in the figures for horses 318, 319, 323 and 325.

The serumglobulin content was maintained high and fairly constant during active antitoxin production. At times there was a tendency for the serumglobulin to follow quantitatively the potency variations; at other times, the increase was independent of the antitoxin changes (horse 326, 5/19/07 and 6/13/07). With horse 325, for instance, the serumglobulin did not increase for a rise in potency from 150 to 250 units (7/2/07 to 9/17/07) in the course of the regular bleedings, but was diminished from 6.09 to 5.39 gms. per 100 c.c. plasma. Subsequently when the lowering of the serumglobulin content was accompanied by a corresponding drop in the antitoxic potency, interruption of the regular "full" bleedings failed to check the diminution of either potency or serumglobulin.

In the course of immunization, then, there is a marked increase (even over 100 per cent.) in the serumglobulin content of the blood plasma. This increase tends to precede the development of maximum antitoxic potency and is quantitatively independent of the latter. With a diminution of the potency, the serumglobulin increase is reduced, with a tendency for this change to precede the antitoxic variation; then after a slight rise, the content of serumglobulin is maintained at a fairly constant level in spite of repeated "full" bleedings at frequent intervals. The serumglobulin now may or may not be independent of incidental variations in the antitoxic potency. As a rule, it does parallel to some extent the pronounced changes in the antitoxic properties of the plasma.

Changes in the "euglobulin" must be considered because the increase during immunization of the serumglobulin has been stated by Joachim¹⁶ to take place in this fraction. Some discussion of the chemistry of the serumglobulin fractions is desirable.

The serumglobulin is commonly stated to consist of at least two different proteins, termed the "euglobulin" or true globulin, and a more soluble protein, the "pseudoglobulin." The "euglobulin" is supposed to be insoluble in pure water, and accordingly precipitable on dilution or dialysis; it is thrown down also by slight acidification. The "pseudoglobulin" belongs by definition to the group of true albumins, i. e., it is soluble in pure water. It has received the title "pseudoglobulin"

probably because the earlier differentiation was made on the basis of precipitation on saturation of its solution with magnesium sulphate. The separation of the two proteins then should be relatively easy by the dialysis method. It had always been our experience, however, that the ordinary globulin precipitation methods, with the exception of the salting out reactions, are practically without effect on horse serum. On dilution, dialyzation¹⁷ or slight acidification of the natural or previously neutralized (phenolphthalin) horse serum only a very small amount of precipitate is obtained. In practice, investigators have commonly used the fractional precipitation by salting out with ammonium sulphate. A half volume of the saturated ammonium sulphate solution is added to the serum; the precipitate is the "euglobulin." The separation is not sharp at all. The filtrate contains the serumalbumin and "pseudoglobulin"; the latter can be thrown down by the further addition of saturated ammonium sulphate solution to "half" saturation, i. e., until the mixture contains equal volumes of serum and the salt solution. The "euglobulin" may be further purified by redissolving the precipitate in a measured quantity of water and again adding a half volume of the saturated ammonium sulphate solution. In current writings the identity of this "fractioned" euglobulin has been confused with the slight precipitate obtained on dialysis, dilution or slight acidification in spite of the early work of Freund and Joachim.¹⁸

The differentiation of the serumglobulin into "eu" and "pseudo" fractions by the ammonium sulphate salting out was further established by the result of E. P. Pick.¹⁹ This investigator fractioned various antitoxic, agglutinative and other sera and differentiated the antibodies chemically according to the fraction with which these were precipitated. Others²⁰ have also similarly fractioned individual antisubstances of various kinds and reported with which fraction their own particular body was associated. One of us (with Dr. K. B. Collins)²¹ has already shown that no such separation of the agglutinins in poly-agglutinative sera can be made, and has failed to verify Pick's results on antitoxic sera.²² We²³ have, however, found that the more soluble of several fractions of the "pseudoglobulin" are richest in antitoxin per gm. protein. By employing the ammonium sulphate fractioning, Led-

ingham,²⁴ like Joachim, found that the increase in serumglobulin affected the "euglobulin" more than the "pseudoglobulin" fraction, i. e., the great increase in the serumglobulin took place in the non-antitoxic portion. Ledingham's interpretation of his results, as regards this point, seem inconclusive, for he does not appear to grasp the significance and understand the limitations in the salt fractioning of proteins.

We have already stated in an earlier paper that precipitation limits in the salting out of proteins are characteristic only in so far as they should be interpreted as well from the standpoint of the *solubility of the protein in certain amounts of the salt solution of a definite concentration*. Precipitation occurs because the solvent is saturated with the protein, i. e., it can hold no more in solution; with a greater volume for the solvent more precipitated protein will go into solution. Moreover, other kinds of proteins remaining in solution probably affect the precipitation of the less soluble forms. Haslam's²⁵ criterium of purification calls for repeated precipitation at the same volume until the organic N. in the filtrate is constant.

The observations associating the increase serumglobulin with the questionable "euglobulin" fraction have been made by direct precipitation of the serum with half its volume of saturated ammonium sulphate. Where precipitated for purification, the protein has usually been redissolved to the original volume and the same amount of ammonium sulphate solution added as before. Inasmuch as the serumglobulin content may have been doubled as the result of immunization, would not a great part of this increase be found naturally in the "euglobulin" fraction, since solubility of the protein must be considered? The precipitate obtained at "one-third" saturation is not free from antitoxin.²⁶ It may contain the greater part of it when the undiluted serum is precipitated by the addition of a half volume of saturated ammonium sulphate solution.

Ten c.c. of the normal plasma were precipitated at one-third saturation (3.3) by the direct addition of 5 c.c. of the saturated ammonium sulphate solution; again 10 c.c. of the plasma were diluted 33 1-3 c.c. and precipitated by the addition of 16 2-3 c.c. of the saturated ammonium sulphate solution, and finally, a third 10 c.c. of the plasma

were diluted to 66 2-3 c.c. and precipitated with 33 1-3 c.c. of the saturated salt solution. We had then ultimate dilutions to 15, 50 and 100 c.c. The precipitates were allowed to stand for some hours, with occasional shaking, to permit mechanically precipitated material in part to return to solution. The "euglobulin" at the three dilutions was determined as outlined earlier in the paper.

In such an experiment with horse 319 the normal total serumglobulin (4.24 gms. per 100 c.c. plasma) was found to include 2.43, 0.79 and 0.48 gms. of "euglobulin," according as the dilution of the precipitated plasma was 1:1.5, 1:5 or 1:10. When similarly fractioned at the height of immunization the three "euglobulins" were 5.39, 1.78 and 0.98 gms. at the three respective dilutions. Of the increase of 3.66 gms. in the total serumglobulin, about four-sevenths (2.96 gms.) would be contained in the "eu" fraction at the 1:1.5 dilution; 0.99 gms., or about two-sevenths, would be accounted for at the 1:5 dilution, and but one-seventh at the 1:10 dilution. The precipitations were probably influenced by the lowered serumalbumin content of the antitoxic plasma. It will be noted that the "euglobulins" obtained at the three ultimate dilutions have about the ratio 5:2:1 for almost any of the plasma samples analyzed.

A similar behavior on the part of the "euglobulin" at the several dilutions was observed in the analyses for each horse. Most of the "euglobulin" determinations during the routine "full" bleedings were made at the 1:10 dilution only, and these figures probably most fairly represent the content of this protein if such actually exists. Throughout the whole series of experiments, the per cent. increase in the "euglobulin" tended to parallel that of the total serumglobulin.

Because of the unsatisfactory characterization of the "eu" and "pseudo" globulin by the ammonium sulphate fractioning, it seemed of interest to differentiate in some modified way the relative serumglobulin quantitative changes. We have accordingly determined that portion of the serumglobulin precipitated on saturation with sodium chloride. Freund and Joachim state that saturation with NaCl precipitates all the "euglobulin," but that this includes only a part of the product obtained at 3.3 saturation ammonium sulphate. We may consider that

we have as sharp a differentiation as possible between the two serumglobulins; of course, the individuality of each cannot yet be asserted. The NaCl separation probably more truly represents the common conception of the "euglobulin" and "pseuglobulin," though the ammonium sulphate fractioning has usually been employed.

A glance at the table of analyses for any bleeding will show that the serumglobulin precipitated on saturation with NaCl and the ammonium sulphate "euglobulin" are quantitatively different. Accepting for the "euglobulin" the figures obtained with the $(\text{NH}_4)_2\text{SO}_4$ at 1:10 dilution, we found that the sodium chloride gave a precipitate of serumglobulin in the normal plasma that was from two to three times greater than the corresponding fractioned protein.

The changes during the immunization in the saturated NaCl precipitated serumglobulin are significant. With the rapid rise in the serumglobulin content after immunization has been started, there was observed a very considerable increase in the portion of the serumglobulin precipitated on saturation with NaCl. This rise is shown in the record for horses 322, 323 and 327, but was not observed with the others.²⁷ Subsequently there was a sharp fall in the NaCl precipitable serumglobulin. This decrease was maintained far below the absolute and per cent. normal, and tended to vary inversely with the total serumglobulin content; the behavior in this respect is exactly the opposite of the "euglobulin" fractioned with ammonium sulphate, and parallels closely the changes in the serumalbumin.

For example, the serumglobulin precipitated on saturation with NaCl, in the normal plasma of horse 325 was 0.91 gm. per 100 c.c. plasma of 24.7% of the total serumglobulin (3.68 gms.) with the preliminary rise of 36.7% in the total serumglobulin, the NaCl "euglobulin" had increased over 60% above the normal (1.54 gms. per 100 c.c. plasma) and to 30.6% of the total serumglobulin. The serumglobulin precipitable on saturation with NaCl then sharply fell; with the coincident maxima of antitoxic potency and total serumglobulin content, this NaCl "euglobulin" was only 0.49 gms. (per 100 c.c. plasma) or 7.1% of the total globulin. At the same time the $(\text{NH}_4)_2\text{SO}_4$ "euglobulin" had increased from a normal of 0.42 gms. to 1.00 gm. per 100 c.c. plasma.

The variations in the content of serumalbumin, during immunization, have already been described in detail by Atkinson and by Ledingham. We have also found the curious and characteristic diminution in the content of the plasma in this protein. The minimum content is reached as a rule somewhat subsequent to the time of the serumglobulin

and antitoxic maxima; it occurred usually during the period of the routine bleedings. While the greatest increase observed in the total serumglobulin was about 114 per cent., the serumalbumin may be reduced to less than 20 per cent. of the normal—a diminution from 2.80 gms. to 0.52 gm. being observed in the case of horse 319. The course of the plotted curve of the serumglobulin then was roughly the reverse of that of the total serumglobulin; the serumalbumin, however, tended to return to the higher level when the horse had been in use for several months as an antitoxin producer (319, 323, 325).

The behavior of the NaCl "euglobulin" and the serumalbumin suggests that these proteins are sacrificed in order to compensate for the great absolute increase in the more soluble serumglobulin with which the anti-properties are associated. Possibly because of the increasing viscosity, there must be a physiological limit to the amount of protein which can be carried in the blood plasma. Some 80 odd determinations have indicated that this limit is attained when the protein content is between 10 and 11 gms. per 100 c.c. plasma.*

The changes in the fibrinogen were often sudden and there was no apparent association with antitoxic potency, serumglobulin content or with bleeding. Analyses gave figures for fibrinogen of from 0.21 gm. to 1.0 gm. per 100 c.c. plasma, with variations in the individual horse of about 0.5 gm.

SUMMARY

Gravimetric determinations were recorded for the total and several individual proteins (in the sodium oxalate plasma) fractionated with ammonium sulphate and sodium chloride. At precipitation, the plasma salt mixture had been diluted to a final volume of 10 times the amount of plasma employed. Coagulations were on aliquot portions of filtrates, and the individual protein constituents (except serumalbumin) were calculated by difference.

The 11 horses had been subjected to simultaneous immunization against diphtheria and tetanus toxins, each horse being subsequently

* Moll²⁸ has suggested that this serumalbumin is chemically transferred over into the serumglobulin during immunization. This statement is based on his heat and alkali transformation of albumin into globulin (?). Are we to accept, however, that the NaCl "euglobulin" might similarly be changed over into the more soluble serumglobulin?

continued on the toxin to which it responded best. Test bleedings of about 500 c.c. only were made until maximum antitoxic potency (with almost coincident greatest variation in the contents of the several proteins) had been attained; routine bleedings of 4 to 10 liters for antitoxin production were then instituted.

The two refractory, one medium and the eight horses yielding a highly potent antitoxic plasma, all showed an increase of from 40 to 114 per cent. in the total serumglobulin. For the refractory animals, this increase was 59.3 and 87.9 per cents. In one refractory and one high horse, the serumglobulin maximum preceded the highest concentration in antitoxin. In seven of the horses, the greatest increase in the total serumglobulin was coincident with the maximum antitoxic potency. The serumglobulin increase, however, tended relatively to precede that of the antitoxin. In the two other horses, both maxima also were observed together; no "intermediate" bleedings were made in these two instances. The greatest absolute increase in the serumglobulin was observed in the most potent plasma obtained in the series; the second place, however, went to a refractory horse. Subsequent to the maxima, the serumglobulin content was maintained at high concentration, in spite of repeated bleedings; it then only roughly paralleled the antitoxic variations in the plasma of the individual animals.

At dilutions of the plasma in the precipitated mixtures of 1:1.5, 1:5 and 1:10, the ammonium sulphate "euglobulin" fraction amounted to about 67-70, 20-24 and 10-15 per cents., respectively, of the total serumglobulin in both the normal and the antitoxic plasma. In an 850 unit plasma an increase in the "euglobulin" over the normal per cent. was observed, but the high content of protein in this plasma probably influenced the precipitation limits. The influence of the protein concentration is indicated by the different percentages for the "euglobulin" obtained for the three dilutions of 1:1.5, 1:5 and 1:10. The "euglobulin" then was not increased to a greater extent than the "pseudoglobulin," as the result of immunization, as has at times been maintained.

The "euglobulin" precipitated by saturating the plasma with NaCl (at ultimate dilution of the plasma 1:10) was much greater in normal plasma than the $(\text{NH}_4)_2\text{SO}_4$ "euglobulin" at the same dilution. There

was a tendency in early immunization for this NaCl "euglobulin" to increase along with the total serumglobulin; it rapidly diminishes, however, until at the height of immunization and maximum serumglobulin concentration, it may have reached less than half the normal absolute amount.

The serumalbumin was diminished a third to a half the normal along with the serumglobulin increase. Subsequent to the antitoxic and serumglobulin maxima, figures as low as a fifth the original serumalbumin content have been noted.

It is suggested that this diminution of the NaCl "euglobulin" and the serumalbumin is a physiological compensation for the greater viscosity of the plasma, because of the increase in the more soluble serumglobulins.

No characteristic alteration in the fibrinogen of the plasma was observed during immunization. Individual variations up to 0.5 gm. per 100 c.c. plasma have been regarded.

The influence of repeated bleedings does not essentially influence the protein changes induced by immunization. These remarkable regenerative processes are worthy of note.

The results of our investigation indicate that in "forced" immunization, the same characteristic quantitative changes can occur in the blood proteins of both refractory horses and of those yielding a highly potent antitoxic plasma. We cannot conclude, however, that the serumglobulin increase does not represent an accumulation of antitoxin, at least in part; it is possible that other antibodies may be formed either prior to or along with the specific antitoxin²⁹ and that these may constitute a portion of the increase in the more soluble serumglobulin with which protein such substances are associated.

¹ Seng: *Zeitschr. f. Hyg.* xxxi, p. 513, 1899.

² Joachim: *Arch. f. d. ges. Physiol.*, xciii, p. 558, 1903.

³ Atkinson: *Journ. of Exper. Med.*, v, p. 67, 1901.

⁴ Ledingham: *Journ. of Hygiene*, vii, p. 65, 1907.

⁵ Moll: *Zeitschr. f. exp. Path. u. Ther.*, iii, p. 325, 1906.

⁶ Glaessner: *Zeitschr. f. Exp. Path. u. Ther.*, ii, No. 1, 1905.

⁷ Atkinson: *Journ. of Exper. Med.*, v, p. 47, 1901.

⁸ Under the classification of the proteins adopted by the American Physiological Society and the American Society of Biological Chemists, the greater part of the so-called serumglobulins are, at least for the horse, to be regarded as albumins.

⁹ Ledingham: *Loc. cit.*

¹⁰ With the more soluble or "albumin" type of the serumglobulin.

¹¹ Mellanby: *Proc. Royal Soc., B*, lxxx, p. 399, 1908.

¹² It was originally Dr. Park's intention to report this immunization experiment in detail. The present paper contains a description of the experiment and the results only in so far as is necessary to make our own report complete.

¹³ The degree of saturation, as here expressed, indicates a concentration in 10 c.c. of the precipitated mixture of 2.9 c.c. of saturated ammonium sulphate solution. The misconceptions that arise from the use of "per cent. saturation" and even from "third saturation" and "half saturation" have already been discussed in a previous paper. Cf. Banzhaf and Gibson: *Journ. of Biol. Chem.*, iii, p. 254, 1907.

¹⁴ By "serumalbumins" is meant the protein material commonly known as such and consisting probably of more than a single individual substance. Similarly, the "serumglobulins" refer to the remaining proteins of the plasma, excepting the fibrinogen.

¹⁵ The analyses for the plasma obtained at the first "full" bleeding are discussed with the earlier or "test" bleedings inasmuch as the horses had not previously been subjected to the severer hemorrhage.

¹⁶ Joachim: *Loc. cit.*

¹⁷ Long continued dialysis may lead to denaturalization and precipitation of otherwise soluble proteins, more than 48 hours is hardly necessary for the separation of typical albumins and globulins.

¹⁸ Freund and Joachim: *Zeitschr. f. physiol. Chem.*, xxxvi, p. 407, 1902.

¹⁹ Pick: *Beiträge z. chem. Physiol. u. Path.*, i, p. 351, 1901.

²⁰ Fuld and Spiro: *Zeitschr. f. physiol. Chem.*, xxxi, p. 133, 1900; Landsteiner: *Centralbl. f. Bakt., xxvii, Abt. I*, p. 357, 1900; Jacobi: *Beitr. z. chem. Physiol. u. Path.*, I, p. 51, 1901; Cathcart: *Journ. of Physiol.*, xxxi, p. 497, 1904; Glaessner: *Beiträge z. chem. Physiol. u. Path.*, iv, 79, 1904; Simon, Lamar and Bispham: *Journ. of Exper. Med.*, viii, p. 651, 1906; Opie and Barker: *ibid.*, ix, p. 207, 1907.

²¹ Gibson and Collins: *Journ. of Biol. Chem.*, iii, p. 233, 1907.

²² Ledingham (*Loc. cit.*) also states that the diphtheria antitoxin in goat serum is not invariably linked to the euglobulin fraction as maintained by Pick.

²³ Banzhaf and Gibson: *Loc. cit.*

²⁴ Ledingham: *Loc. cit.*

²⁵ Haslam: *Journ. of Physiol.*, xxxii, p. 267, 1905.

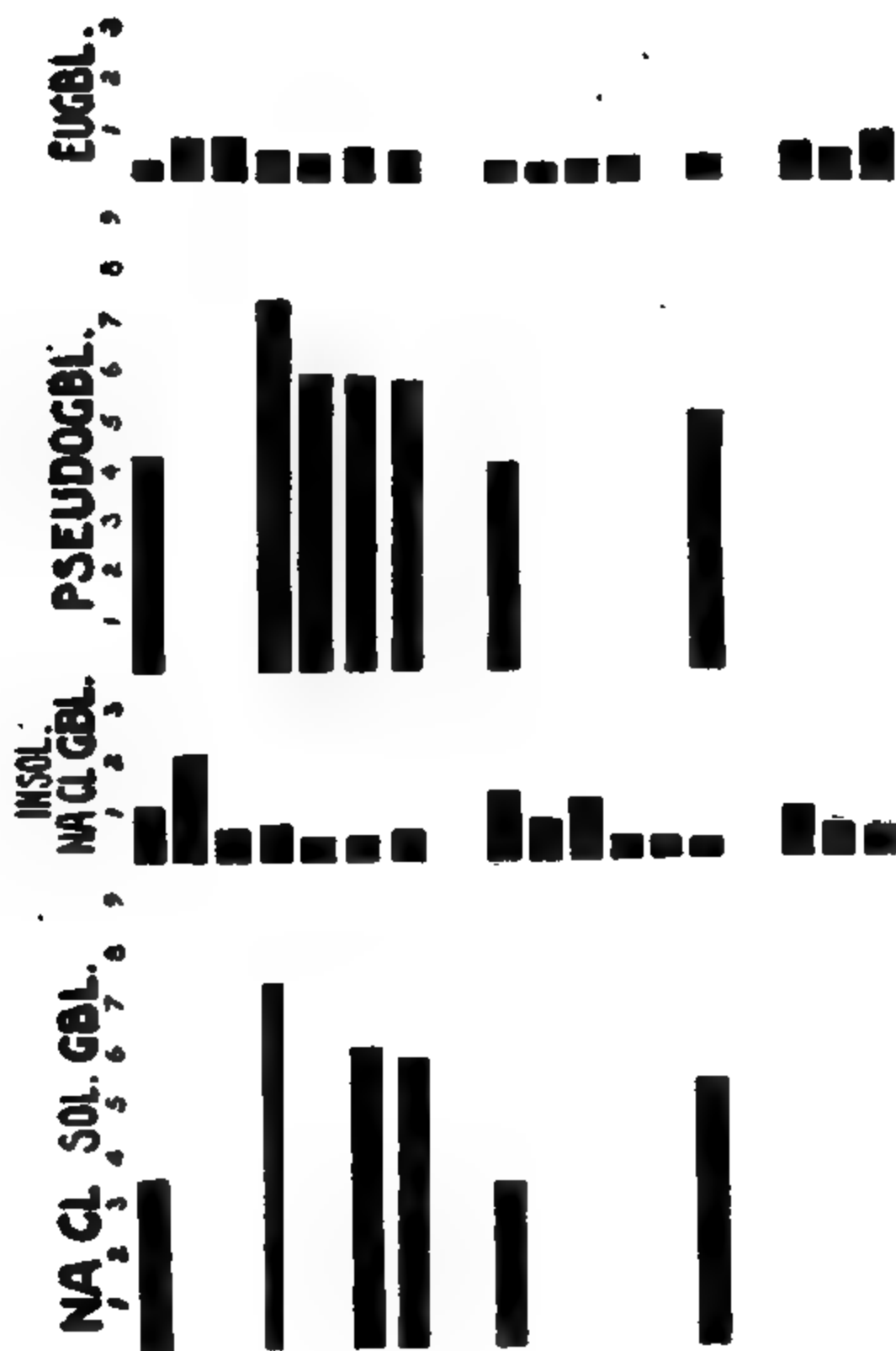
²⁶ Brieger: *Festschr. f. R. Koch*, Jena, 1903. See also Ledingham: *Loc. cit.*

²⁷ On horses 318 and 319 there were no intermediate test bleedings.

²⁸ Moll: *Beiträge z. chem. Physiol. u. Path.*, iv, p. 363, 1904.

²⁹ Dr. K. R. Collins (*Journ. of Exper. Med.*, x, p. 529, 1908) has shown that group and specific bacterial agglutinins can be developed by immunization against yeasts, enzyme preparations and nucleins; these experiments then afford actual proof of the development of widely different anti-substances during immunization. A similar stimulation to production of numerous antibodies in addition to precipitins and specific antitoxins, probably occurs on immunizations with bacterial toxins.

HORSE N° 323		ALB. & GBL.										SER. GBL.			SER. ALB.			FIBR.	
DATE	BLIN. UNITS	1	2	3	4	5	6	7	8	9	10	1	2	3	1	2	3	1	2
2-26	1	NORMAL																	
3-25	2	500																	
4-29	3	850-15T																	
5-10	4	5500																	
6-17	5	5000																	
9-12	14	4750																	
11-19	21	6250																	
HORSE N° 320																			
1-31	1	NORMAL																	
3-11	2	200																	
4-1	3	3500-2T																	
4-30	4	500-50T																	
6-6	5	500-90T																	
6-17	6	300-100T																	
HORSE N° 324																			
3-25	1	NORMAL																	
4-30	2	250-1T																	
6-19	6	6250																	



GRAPHIC REPRESENTATION OF PROTEIN DISTRIBUTION.

PER CENT

ALB. & GBL.

SER. GBL.

SER. ALB.

NA CL SOL. GBL.

NA CL INSOL.GBL.

PSEUDOGBL.

EUGBL.

ALB. & GBL.

SER.GBL.

SER. ALB.

FIBRINOGEN

NA CL SOL. GBL.

NA CL INSOL. SOL.

PSEUDOGBL.

EUGBL.

ALB. & GBL.

SER. GBL.

SER. ALB.

FIBRINOGEN

NA CL SOL. GBL.

NaCl INSOL. GBL.

PSEUDOGBL.

EUGBL.

ALB. & GBL.

SER. GBL.

SER. ALB.

FIBRINOGEN

NA CL SOL. GDL.

NACL INSOL. GBL.

PSEUDOGBL.

EUGBL.

PROTEIN VARIATION DURING IMMUNIZATION*

Horse No. 318

Date of Bleeding.	Last Amt. Dip. Toxin Inj. About Seven Days Before Date of Bleeding.	Last Amt. Tet. Toxin Inj. About Seven Days Before Date of Bleeding.	Dip. Units per c.c.	Tet. Units per c.c.	Amount Plasma Recovered from Bleeding.	At Dil. 1:10 Total Protein.	At Dil. 1:10 Fibrinogen.	At Dil. 1:10 Serum Albumin.	At Dil. 1:10 Serum Globulin.	At Dil. 1:10 NaCl Insol. Globulin.	At Dil. 1:1.5 Eu Globulin.	At Dil. 1:5 Eu Globulin.	At Dil. 1:10 Eu Globulin.
1-24-07	Normal				400								
3-11-07	375	160	600	1	400	9.35	0.48	2.83	4.89	1.68	3.26	1.18	0.65
4- 2-07	475	250	550	10	7300	8.97	0.32	1.97	6.90	1.44	4.68	1.78	0.98
4-10-07	600	375	500	70	7700	8.32	0.42	1.95	6.70	0.90	4.84	1.80	0.96
4-24-07	Dip.	900	400	110	7850	8.04	0.56	1.84	6.06	0.45			0.96
5-14-07	Toxin	1200	200	125	7060	8.89	0.68	1.47	6.01	0.84	4.31	1.55	0.79
5-20-07	Discon- tinued.		125	160	6070	8.15	0.53	0.97	7.24	0.55	4.89	1.80	0.96
6- 8-07		1000	50	300	5000	8.18	0.31	1.08	6.79	0.73			0.99
7- 3-07		1200	10	250	6400	7.90	0.27	1.27	6.36	0.70	4.82	1.79	0.96
7-10-07		Tet.	4	200	6300	7.54	0.34	1.25	5.95	0.57			
9- 6-07	400†	Toxin	200		400	8.11	0.40	1.30	6.41	0.75			0.99
9-25-07	560	Discon- tinued.	400		5900	8.20	0.35	1.34	6.51	0.60	4.77	1.75	0.97
10- 9-07	600		450		6000	8.19	0.27	1.50	6.42	0.58			1.00
10-19-07	650		500		5900	8.30	0.30	1.66	6.34	0.62			0.96
10-30-07	700		550		6100	8.60	0.21	1.86	6.53	0.59			0.93
11- 8-07	750		550		6300	8.77	0.24	1.88	6.65	0.75			1.00
11-19-07†	750		575		6000	8.72	0.30	1.82	6.60	0.73	4.56	1.68	0.87

* Analyses are given as grams coagulable protein per 100 c.c. of plasma.

† Subsequent bleedings were as follows: 11-27-07, 450 units, 6,100 c.c.; 12-6-07, 450 units, 5,850 c.c.; 12-16-07, 450 units, 5,800 c.c.; 12-27-07, 400 units, 6,200 c.c.; 1-6-08, 350 units, 6,200 c.c., and 1-7-08, 200 units, 8,000 c.c. The horse died while bleeding on 1-7-08.

‡ Diphtheria toxin injections resumed August 14, 1907.

PROTEIN VARIATION DURING IMMUNIZATION*

Horse No. 323

Date of Bleeding.	Last Amt. Dip. Toxin Inj. About Seven Days Before Date of Bleeding.	Last Amt. Tet. Inj. About Seven Days Before Date of Bleeding.	Dip. Units per c.c.	Tet. Units per c.c.	Amount Plasma Recovered from Bleeding	At. Dil. 1:10 Total Protein.	At Dil. 1:10 Fibrinogen.	At Dil. 1:10 Serum Albumin.	At Dil. 1:10 Serum Globulin.	At Dil. 1:10 NaCl Insol. Globulin.	At Dil. 1:1.5 Eu Globulin.	At Dil. 1:5 Eu Globulin.	At Dil. 1:10 Eu Globulin.
2-16-07	Normal				400	8.79	0.74	2.33	4.60	1.16	2.21	0.68	0.37
3-25-07	110	70	50		400	10.49	0.71	1.98	6.07	2.06	4.06	1.45	0.82
4-29-07	400	525 Tet.	850	5	400	9.47	0.80	0.78	9.00	0.66	4.50	1.53	0.85
5-10-07			550		6000	8.29	0.39	0.66	8.01	0.70	3.29	1.22	0.67
6-17-07	375	Toxin	500		7700	8.36	0.29	1.45	6.45	0.46	2.85	1.00	0.57
7-2-07	475	Discontinued.	500		8000	8.16	0.32	1.33	6.74	0.64			
7-10-07	500		450		6200	7.92	0.32	1.25	6.41	0.61	3.99	1.39	0.75
7-18-07	550		450		6200	8.08	0.48	1.14	6.46	0.79	3.80	1.30	0.71
7-29-07	600		450		6200	8.00	0.40	1.24	6.36	0.41			0.73
8-9-07	650		425		6100	8.20	0.47	1.30	6.30	0.51			0.69
8-17-07	700		425		6200	8.15	0.44	1.32	6.41	0.58			0.68
8-27-07	750		425		6300	8.30	0.50	1.25	6.46	0.46			0.70
9-3-07			425		6000	8.35	0.55	1.30	6.50	0.50			0.67
9-12-07	800		475		6250	8.45	0.46	1.33	6.47	0.47			0.68
9-19-07	800		500		5200	8.30	0.50	1.40	6.59	0.52			0.70
9-28-07	800		550		6400	8.50	0.45	1.55	6.25	0.50			0.65
10-9-07	800		575		6100	8.65	0.50	1.75	6.20	0.55			0.55
10-19-07	850		550		6200	8.71	0.53	2.00	6.15	0.53	2.87	0.99	0.57
10-30-07	850		625		6100	9.10	0.53	2.18	6.00	0.48	2.92	1.01	0.55
11-8-07	850		600		6300	8.56	0.51	2.00	6.57	0.64	3.15	1.12	0.61
11-19-07†	850		625					1.73	6.32	0.57			

* Analyses are given as oxalate protein per 100 c.c. plasma.

† as follows: 11-27-07, 600 units, 5,900 c.c.; 12-6-07, 600 units, 5,800 c.c.; 12-16-07, 600 units, 5,900 c.c.; 1-6-08, 400 units, 7,700 c.c.; 1-14-08, 400 units, 7,500 c.c.; 1-22-08, 400 units, 8,000 c.c.; 1-31-08, 400 units, 7,800 c.c.; 2-7-08, 425 units, 8,000 c.c.; 2-13-08, 300 units, 8,000 c.c.; 3-12-08, 300 units, 8,000 c.c. There were 275 liters of whole blood. The horse died suddenly on 7-30-08; toxin had not been given after 3-12-08.

PROTEIN VARIATION DURING IMMUNIZATION*

Horse No. 319

Date of Bleeding.	Last Amt. Dip. Toxin Inj. About Seven Days Before Date of Bleeding.	Last Amt. Tet. Toxin Inj. About Seven Days Before Date of Bleeding.	Dip. Units per c.c.	Tet. Units per c.c.	Amount Plasma Recovered from Bleeding.	At. Dil. 1:10 Total Protein.	At Dil. 1:10 Fibrinogen.	At Dil. 1:10 Serum Albumin.	At Dil. 1:10 Serum Globulin.	At Dil. 1:10 NaCl Insol. Globulin.	At Dil. 1:1.5 Eu Globulin.	At Dil. 1:5 Eu Globulin.	At Dil. 1:10 Eu Globulin.
1-24-07	Normal				400								
3-11-07	375	160	600	1	400	9.32	0.43	2.80	4.24	1.29	2.43	0.79	0.48
4- 2-07	475	250	550	2	7100	8.55	0.33	0.99	7.90	1.10	5.39	1.78	0.98
4-10-07	600	375	525	4	8000	8.51	0.43	1.17	7.05	0.82			1.00
4-18-07	650	Tet.	550		7850	7.85	0.52	1.25	6.83	0.50	5.15	1.89	1.00
4-27-07	700	Toxin	575		5600	8.66	0.71	0.87	6.46	0.69			0.88
5- 7-07	700	Discon-	550		8000	8.02	0.61	0.89	7.06	0.69	5.75	2.00	1.10
5-14-07	750	tinued.	550		8700	8.20	0.82	0.81	6.60	0.49			0.90
5-23-07	750		550		7900	8.66	0.74	0.52	6.86	0.57			0.98
6- 1-07	850		550		6400	8.86	0.76	0.75	7.37	0.67			0.99
6-10-07	850		550		6250	8.70	0.58	0.67	7.35	0.56			1.02
6-17-07	900		525		5950	8.44	0.56	0.90	7.45	0.98			0.98
6-27-07	900		525		5700	8.65	0.65	1.21	6.98	0.88			0.89
7-10-07	900		500		5900	8.82	0.41	1.30	6.79	1.00			0.98
									7.11	1.05			1.00
Horse No. 320													
1-31-07	Normal				400								0.40
3-11-07	260	100	20		400	8.50	0.47	2.60	4.52	1.30	2.03	0.72	0.40
4- 1-07	500	350	35	2	400	10.14	0.34	1.57	6.46	0.82	1.80	0.60	0.34
4-30-07	600	1050	50	50	400	9.63	0.74	1.32	8.48	1.20	2.12	0.74	0.41
6- 8-07	Dip.	1350	50	90	5600	8.55	0.49	1.22	7.67	0.45	2.61	0.90	0.50
6-17-07	Toxin	1100	30	100	7100	7.64	0.41	1.35	6.71	0.40			0.49
6-26-07	Discon-	1100	10	90	6200	7.70	0.48	1.59	5.64	0.35	2.58	0.90	0.49
	tinued.							1.61	5.61	0.27	2.69	0.93	0.51

* Analyses are given as grams coagulate protein per 100 c.c. of plasma.

PROTEIN VARIATION DURING IMMUNIZATION *

Date of Bleeding.	Last Amt. Dip. Toxin Inj. About Seven Days Before Date of Bleeding.	Last Amt. Tet. Toxin Inj. About Seven Days Before Date of Bleeding.	Dip. Units per c.c.	Tet. Units per c.c.	Amount Plasma Recovered from Bleeding.	At Dil. 1:10 Total Protein.	At Dil. 1:10 Fibrinogen.	At Dil. 1:10 Serum Albumin.	At Dil. 1:10 Serum Globulin.	At Dil. 1:10 NaCl Insol. Globulin.	At Dil. 1:1.5 Bu Globulin.	At Dil. 1:5 Bu Globulin.	At Dil. 1:10 Bu Globulin.
Horse No. 324**													
3-25-07	100	70	¼		400	7.88	0.45	1.88	5.55	0.98	3.72	1.37	0.74
4-30-07	400	525 Tet.	25	1	400	10.43	0.76	0.83	8.84	0.63	3.30	1.18	0.65
5-31-07	725	Toxin	125		400	9.49	0.61	0.64	8.24	0.56			0.84
6-19-07	900	Discontinued.	125		8000	9.06	0.42	0.93	7.71	0.53	4.79	1.75	0.98
Horse No. 325													
2-26-07	Normal				400			2.61	3.68	0.91	1.79	0.76	0.42
3-25-07	110	70	4		400	7.78	0.50	2.25	5.03	1.54	3.78	1.39	0.77
4-30-07	400	525	300	4	400	8.65	0.59	1.16	6.90	0.49	5.15	1.84	1.00
5-29-09		1000 Tet.	200	25	400	7.74	0.54	1.41	5.79	0.50	4.72	1.74	0.98
7- 2-07	550	Toxin	150		6800	8.11	0.39	1.63	6.09	0.90			1.00
7-10-07	600	Discontinued.	150		5300	8.45	0.35	2.31	5.79	0.82			0.99
9- 3-07	1050		200		7300	7.95	0.40	2.10	5.45	0.90			0.97
9-17-07	1200		250		6100	8.10	0.41	2.30	5.39	0.84			0.95
9-28-07	1200		200		5350	7.90	0.36	2.29	5.25	0.80			0.93
10- 9-07	1200		200		5400	8.00	0.38	2.35	5.27	0.70			0.80
10-19-07	1300		175		5600	7.80	0.40	2.41	4.99	0.65			0.85
10-30-07	1300		150		1100	7.86	0.36	2.44	5.06	0.58	4.20	1.54	0.81
11- 8-07			125		400	7.61	0.30	2.55	4.76	0.64			0.67
11-19-07			75		400	6.95	0.55	2.29	4.11	0.37	2.37	0.81	0.52
Horse No. 326													
3- 6-07	Normal				400	7.15	0.33	2.82	4.00	1.09	2.06	0.77	0.42
4- 1-07	100	50	5		400	8.47	0.46	2.38	5.63	0.96	3.52	1.26	0.70
4-30-07	350	400 Tet.	600	1	8000	9.41	0.64	1.16	7.61	0.42	4.68	1.66	0.90
5-10-07	400	Toxin	600		8000	8.08	0.72	1.44	5.92	0.63	3.87	1.34	0.72
5-18-07	450	Discontinued.	600		7800	8.73	0.79	0.98	6.96	0.61	3.78	1.29	0.70
5-29-07	500		600		8000	8.73	0.74	0.97	7.02	0.74	3.93	1.38	0.72
6-13-07	500		550		5600	10.52	0.68	0.61	9.23	1.01	6.46	2.38	1.30

* Analyses are given as grams coagulable protein per 100 c.c. of plasma.

**Immunization started 2-26-07.

THE FURTHER SEPARATION OF ANTITOXIN FROM ITS ASSOCIATED PROTEINS IN HORSE SERUM

By EDWIN J. BANZHAF

The literature concerning means of purification of anti-bodies and their chemical characteristics has been thoroughly reviewed by Gibson,¹ Ledingham,² Banzhaf and Gibson,³ and Brieger and Kraus.⁴

Stark⁵ was the first to report that by heating for one hour at 56° C., ovalbumin could be converted into a body, which, because of its precipitation and solution reactions, and its composition, was obviously a globulin. Later Noll⁶ showed the same to be true of albumin in rabbit, dog and horse serum.

My experiments were to ascertain the resulting conditions after heating antitoxic horse serum, citrated plasma and Gibson's concentrated and partially purified antitoxic globulin solution.

Heating an antitoxic serum prepared by the Gibson method¹ caused an elimination of 23 per cent. protein and an increase of antitoxic units per gram protein of 30 per cent. over the native serum. A number of specimens of the same antitoxic serum were heated for from 6 to 72 hours in closed containers, at a temperature of 57° C. After cooling to room temperature, the sera were saturated with sodium chloride and brought up to a dilution of 1:10 with saturated sodium chloride solution. Twelve hours later the resulting precipitations were filtered off. Potency tests on these filtrates showed a loss of 5 per cent. after heating 6 hours, and an increasing loss up to 22 per cent. after heating 72 hours. The protein converted into an insoluble condition (in saturated sodium chloride solution) was 30 per cent. for the 6-hour period, increasing up to 48 per cent. for the 72-hour period. The increase

¹*Journal of Biolog. Chem.*, i, p. 161, 1906.

²*Journal of Hyg.*, vii, p. 65, 1907.

³*Journal of Biolog. Chem.*, iii, p. 253, 1907.

⁴*Berl. klin. Woch.*, xlv, p. 946, 1907.

⁵*Zeitschr. f. Biol.*, xl, p. 494, 1900 (new series, vol. 22).

⁶*Hofmeister's Beiträge*, iv, p. 563, 1904.

of antitoxic units, per gram protein, was 35 per cent. after 6 hours' heating, increasing up to 53 per cent. after 48 hours.

Owing to the larger per cent. destruction of antitoxin at the 72-hour heating than the per cent. increase converted, the potency per gram protein represented only 52 per cent. increase over the native serum. On separating the remaining unconverted albumin from this series, the increase of antitoxic units, per gram protein, was 60 per cent. after 6 hours' heating, increasing to 78 per cent. after 48 hours. The 72-hour heating showed an increase of 73 per cent. over the native serum.

Citrated plasma under the same conditions gave practically the same results. Gibson's antitoxic globulin solution (blood alkalinity) containing only that globulin soluble in saturated sodium chloride solution was heated under the same conditions. The potency loss was 5 per cent. for the 6-hour period, and an increasing loss up to 23 per cent. for the 72-hour period. The soluble globulin converted into an insoluble condition (in saturated sodium chloride solution) was 30 per cent. after 6 hours' heating, increasing to 47 per cent. after 72 hours. The increase of antitoxic units, per gram of protein, was 37 per cent. after 6 hours' heating, increasing to 54 per cent. for the 24 hours. Here again the 72-hour heating period caused a larger per cent. destruction of antitoxin than per cent. globulin converted into an insoluble condition (in saturated sodium chloride solution), representing only an increase of 46 per cent., per gram protein, over Gibson's antitoxic globulin solution.

This work is being continued, for it is believed to be practically and scientifically important, and may throw some light on the chemical characteristics and the nature of antitoxins.

TABLE I
HORSE 359 CITRATED PLASMA 475 UNITS PER C.C

Number of Hours Heated.	Degrees of Heat.	% Coagulable Protein Minus Fibrinogen.	% Coagulable Albumin.	% Coagulable Protein Soluble in Sat. NaCl Sol.	% Protein Converted into Insoluble in Sat. NaCl Sol.	Units per Gram Coagulable Protein.	% Increase of Antitoxin per Gram Protein.	% Coagulable Protein Soluble in NaCl Sol. minus Albumin.	Units per Gram Coagulable Protein.	% Increase of Antitoxin per Gram Protein.	Units per Gram Coagulable Protein.	% Increase of Antitoxin per Gram Protein.	% Loss of Antitoxin on Heating.
6	57°	7.03	.97	6.36	21.85	6,756	10.53	5.39	6,756	30.43	6,756	30.43	5.26
8	57°	7.03	.77	4.97	24.37	7,468	34.01	4.20	9,054	58.58	8,812	58.58	5.26
15	57°	7.03	.69	4.81	27.04	9,355	38.46	4.12	9,355	61.67	10,922	61.67	5.26
18	57°	7.03	.67	4.64	29.87	9,698	43.54	3.97	9,698	67.77	11,335	67.77	5.26
24	57°	7.03	.65	4.46	35.69	9,865	46.01	3.81	9,865	70.92	11,548	70.92	7.36
48	57°	7.03	.61	4.09	42.92	10,391	53.80	3.48	10,391	80.75	12,212	80.75	10.52
72	57°	7.03	.50	3.63	47.80	10,743	59.01	3.13	10,743	84.42	12,460	84.42	17.89
		7.03	.37	3.32		10,542	56.03	2.95		75.60	11,864	75.60	26.31

TABLE II
HORSE 353 SERUM. 775 UNITS PER C.C.

Number of Hours Heated.	Degrees of Heat.	% Coagulable Protein.	% Coagulable Albumin.	% Coagulable Protein Soluble in Sat. NaCl Sol.	% Protein Converted into Insoluble in Sat. NaCl Sol.	Units per Gram Coagulable Protein.	% Increase of Antitoxin per Gram Protein.	% Coagulable Protein Soluble in Sat. NaCl Sol. minus Albumin.	Units per Gram Coagulable Protein.	% Increase of Antitoxin per Gram Protein.	Units per Gram Coagulable Protein.	% Increase of Antitoxin per Gram Protein.	% Loss of Antitoxin on Heating.
		7.39	1.50	7.20		10,487	2.63	5.70	10,487	2.63	10,487	29.64	
6	57°	7.39	.81	5.19	27.91	10,763	35.03	4.38	13,596	60.00	13,596	60.00	5.16
8	57°	7.39	.75	5.02	30.27	14,161	39.61	4.27	16,780	64.14	16,780	64.14	5.16
15	57°	7.39	.74	4.82	33.05	14,641	43.42	4.08	17,214	69.43	17,214	69.43	6.45
18	57°	7.39	.73	4.73	34.30	15,041	46.15	4.00	17,769	72.83	17,769	72.83	6.45
24	57°	7.39	.64	4.44	38.33	15,327	50.32	3.80	18,125	75.56	18,125	75.56	9.67
48	57°	7.39	.56	4.05	43.75	15,765	53.03	3.49	18,412	77.59	18,412	77.59	16.12
72	57°	7.39	.45	3.76	47.77	16,049	52.18	3.35	18,624	72.84	18,624	72.84	22.58
						15,960			18,126		18,126		

TABLE III

PREP. 138 DILUTED WITH EQUAL VOLUME WATER. EACH C.C.-1100 UNITS

Number of Hours Heated.	Degrees of Heat.	% Coagulable Protein Soluble in Sat. NaCl Sol.	% Protein Converted to Insoluble in Sat. NaCl Sol.	Units per Gram Coagulable Protein.	% Increase of Antitoxin per Gram Protein.	% Loss of Antitoxin on Heating.
		8.70		12,643		
6	57°	6.05	30.45	17,355	37.26	4.54
8	57°	5.94	31.72	17,676	39.80	4.54
15	57°	5.72	34.26	17,919	41.73	6.81
18	57°	5.55	36.20	18,018	42.51	9.09
24	57°	5.40	37.93	18,518	46.46	9.09
48	57°	4.76	45.28	19,432	53.70	15.90
72	56-57°	4.60	47.12	18,478	46.15	22.72

TABLE IV

PREP. 135 DILUTED WITH EQUAL VOLUME WATER. EACH C.C.-475 UNITS

Number of Hours Heated.	Degrees of Heat.	% Coagulable Protein Soluble in Sat. NaCl Sol.	% Protein Converted to Insoluble in Sat. NaCl Sol.	Units per Gram Coagulable Protein.	% Increase of Antitoxin per Gram Protein.	% Loss of Antitoxin on Heating.
		6.78		7,005		
6	57°	4.88	28.02	9,221	31.63	5.26
8	57°	4.80	29.20	9,375	33.83	5.26
15	57°	4.60	32.15	9,782	39.74	5.26
18	57°	4.40	35.10	9,886	41.12	8.42
24	57°	4.30	36.58	10,116	44.41	8.42
48	57°	3.85	43.21	10,389	48.30	15.78
72	57°	3.53	47.93	10,198	45.58	24.21

TABLE V

PREP. 130 DILUTED 750 UNITS PER C.C.

Number of Hours Heated.	Degrees of Heat.	% Coagulable Protein Soluble in Sat. NaCl Sol.	% Protein Converted to Insoluble in Sat. NaCl Sol.	Units per Gram Coagulable Protein.	% Increase of Antitoxin per Gram Protein.	% Loss of Antitoxin on Heating.
		10.70		7,009		
6	58°	7.14	33.27	9,803	39.86	6.66
8	58°	7.09	33.73	9,873	40.86	6.66
10	58°	7.06	34.01	9,915	41.46	6.66
12	58°	6.94	35.14	10,086	43.90	6.66
14	58°	6.59	38.41	4	46.12	10.00
16	58°	6.49	39.34	10,400	48.38	10.00
18	58°	6.35	40.65	10,629	51.64	10.00
24	58°	6.08	43.17	10,690	52.50	13.33

THE ROUTINE PROCESS FOR THE FURTHER PURIFICATION OF THE ANTITOXIC PROTEINS IN HORSE SERUM FOR THERAPEUTIC USE

By EDWIN J. BANZHAF

The Gibson¹ process of purification and concentration was based on the knowledge that the antitoxin is associated only with those globulins which are soluble in saturated sodium chloride solution. In the average normal horse serum the proteins are distributed as follows: Albumin, 40 per cent.; globulin soluble in saturated sodium chloride solution, 42 per cent.; globulin insoluble in saturated sodium chloride solution, 18 per cent. From this it would seem that about 58 per cent. of the total proteins was eliminated and that a considerable purification of the serum had been effected.

Investigation carried on by Banzhaf and Gibson² showed that the proteins of immunized horses fluctuated considerably. These authors estimated quantitatively the albumins, the pseudoglobulins and euglobulins at various dilutions in eleven normal horses and followed the protein distribution of these horses every week through their entire immunization. Their work showed that the total proteins of the immunized horses were increased, and that this increase was at its highest (20 to 45 per cent.) after about two months' treatment. This was true of all the horses, regardless of the unit content. They also showed that the non-antitoxic proteins, the albumin and euglobulin were greatly diminished, while the pseudoglobulin carrying the antitoxin was greatly increased.

In the average antitoxic horse under treatment about three months the proteins of the serum are distributed as follows: albumin, 12 per cent.; globulin carrying the antitoxin, 78 per cent.; globulin insoluble in saturated sodium chloride solution, 10 per cent. Thus it can be seen that the Gibson process will only eliminate about 22 per cent. non-antitoxic proteins.

Early in the year I experimented with antitoxic sera heated at different temperatures and periods of time and found changes taking place in the proteins which allowed me to further purify the antitoxin. Thus, if I heated antitoxin sera of 600 units per c.c. containing albumin 12 per cent., globulin carrying the antitoxin 78 per cent., globulin non-antitoxic 10 per cent., for from 12 to 15 hours at a time at a temperature of 57° C., a rearrangement of the precipitating characteristics of the proteins resulted; so that now there would be albumin 9 per cent., globulin and all the antitoxin 50 per cent., and globulin non-antitoxic 41 per cent., showing an elimination of 50 per cent. non-antitoxic proteins. The loss of antitoxin on heating at a temperature of 57° C. for from 12 to 15 hours was from 5 to 7 per cent.

The following routine process is based on the results recorded in the tables following the preceding abstract "On the Further Separation of Antitoxin from Its Associated Proteins in Horse Serum." This process has given very satisfactory results and is similar to the original process first outlined by Gibson¹ in this laboratory and later improved by Banzhaf and Gibson.²

For routine work in lots 50 or 60 liters, I do not concentrate more than five or six times in order to keep the total proteins in the finished product below 20 per cent.

Antitoxin plasma, in lots of 50 liters, is heated in a water bath at a temperature of 57° C. for from 12 to 15 hours, cooled to room temperature and diluted with 25 liters of water. Saturated ammonium sulphate is added in the proportion: 3 c.c. saturated ammonium sulphate in 10 c.c. diluted plasma. The resulting fractional precipitate will contain all the euglobulin together with a small amount of antitoxin and pseudoglobulin. This precipitate is taken up in saturated sodium chloride solution and carried on according to Gibson's original process. The filtrate from the above precipitate is measured and brought up to 54 saturation ammonium sulphate, according to the following proportions: 2.4 c.c. saturated ammonium sulphate in 10 c.c. filtrate. The resulting precipitate contains only those globulins soluble in saturated sodium chloride solution together with the antitoxin. The second precipitate is pressed to remove excess of ammonium sul-

phate solution containing the albumin and placed in dialyzing bags. Dialysis is continued until all traces of ammonium sulphate are removed. This requires six to eight days' dialysis in running water. After dialysis is complete, sufficient sodium chloride is added to bring the solution up to 0.08 per cent. The first precipitate, even after carrying it through the Gibson process, will also require six to eight days' dialysis to remove traces of ammonium sulphate.

In our former methods on fractioning and separating, the second precipitate was dissolved in saturated sodium chloride and the proteins containing the antitoxin precipitated from this menstruum with acetic acid. Our reason for doing this was to get rid of as much of the ammonium sulphate as possible before dialysis. Now, however, with greatly improved methods of pressing the precipitate, it can be dialyzed directly, thereby saving the otherwise necessary loss of time, antitoxin and other expenses.

The concentration of antitoxin effected with the method outlined is from five to eight times, depending on the quantity and distribution of the protein in the antitoxic plasma. The loss of antitoxin with this method ranges from 16 to 22 per cent.

Rashes of the urticarial character with little or no accompanying constitutional disturbances are less frequent after the therapeutic administration of this further purified product than with the former products.

¹ Journal of Biolog. Chem. I, p. 161, 1906, and Collected Studies from the Research Laboratory, Vol. I, 1905.

² Journal of Biolog. Chem., III, p. 253, 1907, and Collected Studies from Research Laboratory, Vol. III, 1907.

THE PRODUCTION OF DIPHTHERIA ANTITOXIN DURING THE YEAR 1908

By EDWIN J. BANZHAF

The total amount of antidiphtheritic citrated plasma produced in the laboratory during the year 1908 was 1,584,000 c.c. This amount was recovered from 220 bleedings from 24 horses. The highest potency was about 800 units per c.c.; the lowest about 100 units per c.c.

One million three hundred and eighty-five thousand three hundred c.c. of antitoxic citrated plasma, with an average potency of 320 units per c.c. were refined and concentrated by a fractional method devised in this laboratory.

From this amount were obtained 370,940 c.c. of antitoxic globulin solution, with an average potency of 1,009 units per c.c. The highest potency was 2,100 units and the lowest 600 units per c.c.

The following table shows the production of antitoxin from the individual horses:

Horse No.	Number of Bleedings in 1908.	Citrated Plasma in c.c. Obtained in 1908.	Highest Potency in Units per c.c.	Average Potency in Units per c.c.
318	2	14,200	350	275
323	16	122,400	475	358
333	7	50,800	300	221
334	13	92,350	375	309
335	3	22,250	250	208
336	12	94,000	350	279
338	17	123,470	350	289
340	2	15,500	200	200
342	17	126,100	400	318
343	9	59,700	400	300
344	3	23,100	175	158
345	1	18,000	250	250
346	8	55,100	400	343
347	5	34,100	300	230
348	2	13,700	200	200
349	5	37,700	300	250
350	11	81,200	400	309
351	12	95,850	375	341
352	12	89,000	450	352
353	29	195,470	800	473
354	3	19,350	350	313
355	5	34,450	300	245
356	3	21,500	300	213
359	23	144,710	500	431
24	220	1,584,000		

ROUTINE DIAGNOSIS OF RABIES FOR THE YEAR 1908

ANNA W. WILLIAMS

The following is a comparative table of the number of animals sent to the laboratory for diagnosis during 1908 and the previous two years:

Month.	1906.		1907.		1908.	
	Positive.	Negative.	Positive.	Negative.	Positive.	Negative.
January...	7	1	18	3	12	7
February..	5	1	12	6	20	3
March....	9	1	12	2	25	8
April.....	5	1	9	5	23	6
May.....	12	1	13	3	20	18
June.....	10	4	11	7	22	21
July.....	3	7	10	5	9	26
August....	9	5	19	6	16	33
September.	7	2	21	8	8	21
October....	9	8	14	5	9	7
November.	11	1	24	4	7	11
December.	14	5	18	5	4	9
Total.....	101	37	181	59	175	170

It will be seen that there is a decided drop in the number of rabies cases during the latter part of 1908. This is no doubt due to the active destruction of stray dogs by the Health Department at the beginning of this time.

The technic of our diagnostic work remained the same as that of the previous year, as published in Volume III of these studies.

REPORT ON THE AGGLUTINATION TEST FOR GLANDERS

By MARIE GRUND, M.D.

During the six months from October, 1908, to March, 1909, the sera of 979 horses have been tested at this laboratory for the agglutination reaction with the *Bacillus mallei*.

The results of these tests are as follows:

TABLE I
Dilution in which the sera agglutinated

Dilution.	1-200 or Less.	1-500	1-1000	1-2000	1-5000	1-10,000
No. of Sera..	379	224	196	102	46	32

Eight hundred and nineteen of the sera came from horses in three stables, and of these a detailed report on clinical symptoms, mallein reaction and final disposition has not yet been received. It may be mentioned, however, that out of a total of 390 horses in one of these stables, 150 have been destroyed on clinical evidence. This accords, roughly, with the result of the laboratory test which shows that 43.6 per cent. of the 390 sera agglutinated the *Bacillus mallei* in dilutions of 1-1000 and over.

Of the remaining 160 horses, for which clinical data could be obtained, only two were reported as actually diseased on the slip accompanying the serum; according to the final report, however, 51 showed symptoms of glanders. In four of these which came to autopsy the clinical diagnosis was verified by the bacteriological findings.

Table II shows the agglutination reaction grouped with relation to the clinical symptoms, and, as far as could be ascertained, to the mallein reaction. In quite a number of cases mallein was not used, the diagnosis being based on clinical symptoms and the agglutination test.

In the main these results agree with those obtained in previous years. While with higher dilutions the percentage of error diminishes, it is interesting to note that in two apparently normal cases, in

which the serum agglutinated the *Bacillus mallei* in dilutions of 1/10,000, the mallein test bore out the clinical diagnosis against the laboratory test. The fact that less than 50% of the horses whose serum agglutinated the specific organism in high dilution (2,000 and 5,000) were clinically glandered, while in the doubtful cases the

TABLE II

Dilution.	No. Sera.	Clinical Sympt. of Glanders.	Mallein.		Not Used.	Working.	Destroyed.
200 or less	56	8	1	12	43	*43	9
500	28	7	1	13	14	20	8
1,000	31	13	5	9	17	18	13
2,000	19	8	5	7	7	10	9
5,000	13	5	2	7	4	8	5
10,000	13	11	3	2	8	2	11
Total	160	52	17	50	93	101	55

* Four horses under observation to be re-examined.

mallein test was negative, also would indicate that too much dependence must not be put upon the agglutination reaction as an absolutely diagnostic measure but that it must merely be considered as corroborating the other means of diagnosis.

It may be of interest to state that in addition to the sera from horses, specimens of serum from two cases of glanders in the human subject have been tested, both of which reacted promptly in a dilution of 1/5000.

STUDIES ON INTESTINAL AMEBAS AND ALLIED FORMS

BY

ANNA W. WILLIAMS, M.D., AND CAROLINE R. GURLEY

The study of the intestinal amebas was undertaken by us for the following reasons:

First, because there had been so little minute work done on the life cycle of these forms that the question as to whether a distinct species causes amebic dysentery was unsettled; and, second, because of an apparent resemblance in morphology between certain stages in the life history of amebida and of the vaccine and Negri bodies, it was thought that a better knowledge of the former might throw more light upon the nature of the latter.

Critical Review of Recent Work—The two most recent articles on this subject, one by Walker and the other by Craig, contain almost complete bibliographies and reviews of work done, so it is only necessary for us to review the latest reports.

The majority of studies on the intestinal amebas before the work of Musgrave and Clegg were made on smears, hanging drops, and sections, taken directly from the intestinal contents and from the infected tissues of human beings and of inoculated kittens and puppies. There had been a few more or less unsuccessful attempts made to cultivate these micro-organisms on artificial media, but no extensive culture work was reported until 1904 when the studies of Musgrave and Clegg appeared.

These authors, however, did not describe the minute morphologic changes of their organisms; so, up to the time we began to do our work, Schaudinn was the only one who had attempted to give anything like a full life cycle of these forms. Since then his work has been partially corroborated by Prowazek, Craig and Wenyon.

Walker's article appeared in February, 1908. It contains a pretty complete historical review, but his original studies are somewhat disappointing to us, owing to the fact that they were studies only of the

artificial cultures, and not of the organisms in their natural habitat. And though he states that "caution must be exercised in the construction of a life cycle from development on artificial media, since it is possible that not all stages may occur under these conditions," he proceeds, without making control observations on the organisms in their natural habitat, to construct a life cycle and to describe eight new species from the forty-four cultures he has studied. He states that he has made this classification after a critical examination of the characteristics of the ameboid, the encysted, and the sporulating stages as they occur in cultures, but nowhere does he state definitely what these characteristics are. He has presumably given them in his descriptions of the different species at the end of his article, but on tabulating his eight new species and the one he obtained from the Philippines, according to the characteristics he gives, we can find only differences noted which seem to us to be too slight and variable for use in the determining of new species.

In regard to the technic of his culture work, Walker claims to have devised a new method of study, which he calls "the hanging plate method," which he praises highly, suggesting that it might be used with advantage by bacteriologists. This method *has* been used by bacteriologists for many years under the name of the "hanging mass" method.

Walker's culture work on the whole, that is, the preparation of the media, the isolation of pure cultures, the effects of oxygen, moisture and temperature, is practically only a corroboration of the work of Musgrave and Clegg, published in 1904. We have already found that a number of their statements in regard to media and conditions of growth are not true, at least for the organisms with which we are working. For instance, they state that the culture medium must be slightly alkaline, 1 per cent. to phenolphthalein. All of our cultures grow abundantly on a medium that is 1.5 per cent. *acid* to this indicator. This medium, moreover, is the ordinary nutrient agar, and all previous workers, including Walker, have said that this nutrient medium is not fitted for the growth of amebae because it allows too rich a growth of bacteria. They, therefore, recommend a *special* agar medium containing only a small amount of nutriment. We have found, however, that

all of our organisms grow better in the long run on the nutrient agar than on the special medium.

We have also found, contrary to these other observations, that growth is rapid and abundant at blood heat, if the right kinds of bacteria (those that do not grow rapidly at this temperature) are used as food.

One new point which Walker has given in his technic is designed to isolate a single ameba and obtain a pure growth from it. A fresh agar plate is streaked with material from a previous culture, then covered and inverted on the stage of a microscope. With low magnification the location of a single ameba at the edge of the streak is determined, and its position marked with ink or wax pencil on the bottom of the Petri plate with a V-shaped mark, the apex of which just enclosed the ameba. The plate is then turned right side up, the cover removed, and the V-shaped mark traced with carbolic vaseline on the surface of the agar. This isolates the one organism from the others, as no growth can take place through the carbolic vaseline. We have gotten a method far simpler than this, which will be described below.

The morphologic studies of Walker are chiefly on the living organism. He confesses that the technic of his fixed and stained preparations may be faulty, and that, therefore, his observations of minute changes may possibly be incorrect. He says that in all of his forty-seven cultures reproduction takes place in only two ways; first, by simple binary division, and, second, by what he calls sporulation. In the former case he describes briefly amitotic division in the living ameboid forms, and calls attention to the fact that no other observer has reported anything but amitotic division during the vegetative stage. Schaudinn describes for his *Entameba coli* and autogamous sexual process occurring in the *resting* stage (spore stage) in which the nuclei divide by a primitive mitosis, but no other author, except those corroborating his work, has reported mitotic division in the human parasitic forms. Wenyon has described mitosis in the vegetative stage of an ameba from the intestines of the mouse. Walker states that in one case he found in a specimen from a culture of "*ameba coli*" what appeared to be an anaphase of a mitotic division. Therefore, he thinks it is possible that some forms at certain stages may divide mitotically.

In regard to his reproduction by sporulation, which, as he describes it, is new, his descriptions are again indefinite. He does not define the term sporulation as he uses it, but since he says he has been unable to discover any indications of a sexual process, his spore formation probably corresponds either with the process of schizogony or with that of simple budding; and yet he compares this process with the extrusion of spores described by Schaudinn as occurring in *Entameba histolytica* and which Schaudinn considers part of a sexual process. Walker is of the opinion, however, that if a sexual process exists in this group, it probably takes place "by the interchange of reproductive nuclear matter, or chromidia," which he says functions in sporulation.

The study of the process of encystment in these amebas Walker considers a very important aid in the differentiation of species. The size of the cysts, their contents, and the structure of their walls are all dwelt upon, but, as I have already stated, no marked differences are brought out. The variation in size of a single species is great; the contents are spoken of as coarsely or finely granular and the walls as single or double, scalloped or entire, but he does not state whether these appearances vary with the condition of the medium, the age of the culture, etc., and we have found that they vary greatly under different conditions.

He states positively that no reproductive process takes place within the cyst in cultures and, therefore, thinks that others have been mistaken in describing such a process as occurring under any other condition.

There is no summary and the work, on the whole, leaves our ideas still confused in regard to the life cycle of these forms and adds nothing definite to our knowledge of the relationship between the pathogenic and the non-pathogenic organisms.

Craig², the second worker who has made a lengthy study of amebae in tropical dysentery since Schaudinn's work, reports in his latest work the study of 1,579 cases of amebic dysentery together with those from many normal individuals, and from those suffering from diseases other than dysentery. He however goes back to the older method of studying the organisms in their natural habitat and considers cultural studies

misleading. He thinks Walker's work is of practically no worth in identifying Schaudinn's *histolytica* because the latter studied only the cultural forms, and he insists upon the importance of studying the organisms fresh from the animal; indeed, he considers this quite sufficient for coming to the conclusions that Schaudinn's work is correct in every particular, that he (Craig) has fully proved it, and that, therefore, the cause of amebic dysentery is a distinct species of ameba corresponding to Schaudinn's *Entameba histolytica*. He says that he is able easily to make the diagnosis from the study of the living material alone. If this is true, of course, is it a very important addition to our knowledge of amebic dysentery, as 65 per cent. to 70 per cent. of the Philipinos have been found to contain amebas in their intestinal tract, without apparently having symptoms (for a long time, at least six months), while those with true dysentery may have intermissions in symptoms of one to two months. In a simple attack of diarrhea in the former case, if only harmless amebas are present, the question of diagnosis is extremely important.

However correct Craig's observations are on the fresh material, we cannot accept his results from fixed and stained material since he used what we consider faulty technic in the preparation of his slides. He allows them to air-dry before fixing, while everyone who has worked with amebas knows that air-dried specimens are unfit for minute study. The slides *must be* fixed while moist. One must consider all of his work on stained specimens as almost worthless because of this technic. He states that he has been unable to get good stained specimens from cultures by his method of staining, whereas if he had prepared his smears properly he would probably have had no trouble. He describes the amebas as dividing by simple amitotic division, and they appear to do so in air-dried smears, but in the fixed moist smears we get the beautiful mitotic figures, which we describe in a future paper.

In regard to making a differential diagnosis from the more obvious characteristics of the organisms, as seen in fresh stools, I may say that while observations made under such conditions may not be sufficiently accurate for determining different species, they may indicate that such species exist, and they may practically be of use in making diagnoses.

It must be remembered, however, that all of these grosser characteristics vary markedly in the same species under different conditions, and for this reason we should be cautious in accepting conclusions based on them until much more work has been done. That the conditions in certain dysenteries are distinctly different may be the cause of a difference in certain characteristics of the amebas, especially the characteristics which are spoken of as of diagnostic significance, namely, size, prominence of nucleus motility, differentiated ectoplasm, presence of red blood cells, color and vacules. In our cultures we have found that all of these characteristics vary markedly in the same culture under different conditions, and though this is not proof that amebas do so in the intestines, it is an indication that they may.

ORIGINAL WORK

In order to be able to judge of the worth of all this work we decided that it would be necessary to study various free-living and parasitic amebas, both in artificial cultures and in animals.

We began our work with two cultures. One, a free-living form isolated by us from potato parings, and the other obtained by Dr. Calkins from a human case of dysentery in the Philippines.

Later, Walker kindly sent us three cultures; one obtained from the Philippines from a case of human amebic dysentery, the other two isolated by him from the cat and guinea pig, respectively.

Two more cultures, isolated by us later, were added to these, one from the feces of a kitten and the other from those of a puppy.

CULTURAL WORK

Culture media—We found that all of the seven cultures grew abundantly at 20° C. on ordinary nutrient agar; also on glucose agar, glycerin agar, and a few other agars we happened to have made up at the time of our experimentation. We use for our routine stock cultures of these amebae the ordinary nutrient agar, transferring every one to two weeks, though if the agar is thinly poured so that the cysts dry quickly they may remain alive an almost indefinite time. One plate of cysts has been living for three years at ordinary room temperature in the dark. At ordinary ice-box temperatures, also, these agar cultures

remain alive a long time. The longest time tested is a year, when the cultures were still fully viable.

The bacteria used for their food have been chiefly coli, and dysentery bacilli, but a large number of bacteria isolated from feces have been found to be good food, including *B. typhosus* and *Sp. cholerae*. A few intestinal bacteria and some air bacteria proved to be unsuited at first for their growth, but the amebas quickly became accustomed even to these and grew well later. With some air bacteria they never grew well.

All of the cultures grow very well at blood heat if they are grown with bacteria which do not outgrow them. Under these conditions they grow much more rapidly than at room temperature, and the full-grown organisms are much larger than at a lower temperature. They also may contain many vacuoles and proceed more rapidly with abundant chromidial formation. In short, the intestinal forms appear much more like the organisms found in their original habitat, and there is little doubt but that they may be made to present a very similar if not an exactly identical appearances.

On the whole, our seven cultures grew much more readily than we were led to expect appearances.

We found, too, that it was a comparatively easy matter to obtain a culture from a single ameba by the following simple procedure.

With a fine platinum loop a separate ameba at the edge of an agar plate culture is drawn across the field under a low power towards the periphery of the plate. When it is well separated from the rest of the culture a disc of agar with the ameba in the center is cut out with a sterile knife, following the circumference of the objective. The disc is then lifted with the flat of the knife and transferred to a fresh agar plate. The bacteria for the growth of the ameba are now added in *small numbers* to the disc, and the plate set aside in the dark at room temperature. Usually the culture develops in three or four days. Such "pure" cultures show no minute morphologic differences in the individual amebas from those first isolated from their natural habitat.

The minute morphologic changes of these "culture" amebas will be made the subject of a special paper, since the work was done in con-

junction with Dr. Gary N. Calkins, for whose help and encouragement in the whole work we take this opportunity of expressing our thanks.

Suffice it to say, in regard to the growth and development of all these cultural forms, we have gotten unmistakable appearances of specific mitotic division and of budding in the vegetative stages, and of interesting precystic and intracystic changes which point toward a sexual process, different from any before described. These changes are individual enough to be used as a basis for the making of distinct species in this group. In the formation of chromidium and in budding, these amebas show such a close resemblance to appearances in the Negri and vaccine bodies that a new zest is added to the morphologic study of these latter forms.

It is interesting to note that the two cultures from the Philippines, each isolated from human dysenteric stools, show certain constant morphologic changes which constitute their distinct varieties. These characteristics are: (1) Size. Under similar conditions the one is always distinctly smaller than the other. (2) Shape. The smaller one produces spinous processes on solid media, and stretches itself out in long slender forms. The larger one produces labose pseudopodia on similar culture media and never stretches itself out in slender strings. Both produce labose pseudopodia on fluid and semi-fluid media, but the smaller has more slender forms. The cysts of the larger form are larger and more or less regular in size; those of the smaller are smaller and more irregular in size. Cultures grown from a single ameba show similar characteristics.

Animal Experiments—So far, our animal experiments have been few and rather unsatisfactory as regards minute morphologic study, though we have noted one or two interesting points.

Up to the present only four kittens have been used for experimentation with cultures of amebas. The cultures employed were of three different strains of amebas, one isolated by Miss Wilson from potato parings, one from the intestines of a cat, isolated by Walker (*Am. intestinalis*), and the other, Walker's culture from the Philippines, isolated from a human case of amebic dysentery, each of the two latter grown with *B. coli*, and the former with a yellow air bacillus. The ameba ob-

tained from the potato parings has been shown by Calkins to be *Am. limax*. The kitten inoculated with *Am. intestinalis* showed absolutely no symptoms; while that inoculated with "*Am. coli*" had an attack of typical bloody dysentery. *Ameba limax* also produced a short attack of typical bloody dysentery, clinically, which occurred after each feeding, while the food bacteria alone produced no symptoms. On the cessation of the feedings the kittens gradually recovered.

One, the *Ameba coli* cat, was killed and autopsied after the symptoms had fully disappeared and it showed no lesions.

The *Ameba limax* was recovered from the *Ameba limax* cat, together with a tiny ameba. The *Ameba coli* was not recovered from the *Ameba coli* cat, but from this kitten also a tiny ameba similar to that isolated from the other kitten was obtained.

The *Ameba limax* neither immediately after isolation nor later showed any morphologic differences from the original culture.

We have used five puppies, to each of which was fed agar plate cultures from one of the following strains:

1. Control *B. coli*.
2. *Ameba limax* (transplant 42)
3. *Ameba cobaye* (transplant 44)
4. *Ameba coli* (transplant 51)
5. *Ameba coli* (transplant 36)

The feces were examined several times during the two weeks previous to the feeding and in none were amebas found except in No. 3, whose feces contained on two examinations a small number of amebas, which were isolated and found to be a species distinct from the one fed, namely, *Ameba cobaye*.

All of these puppies, except the control, developed a mucous diarrhoea after three to seven days, which was not hemorrhagic and which contained so few of the amebas that none were found in the hanging drops, or smears; and from only two were the organisms recovered in cultures. The puppies did not seem to be ill during the entire three weeks they were fed.

In regard to the cultivation of the two forms, pathogenic and non-pathogenic, there is no proof, as yet, that any form cultivated corresponds with either of Schaudinn's forms, since no one has made a

minute comparative study of cultural and fresh organisms from the same case. It is still thought by some (Jürgens, for instance) that the true *histolytica* cannot be made to grow on artificial culture media, while Craig states that he has never been able to grow *Entameba coli* on artificial media; he does not give his technic, however, so we are unable to judge of the worth of his observations.

CONCLUSIONS

In regard to our own work six strains, though the specific ameba of amebic dysentery may not be among them, we at least have been able to demonstrate the following points of interest in regard to them:

1. Amebas of the general type found in amebic dysentery are comparatively easy to cultivate on ordinary nutrient media.
2. Such amebas may be easily grown from a single ameba.
3. In the cultural forms definite mitotic division occurs in the vegetative stage as the only mode of binary division.
4. Budding may occur throughout the vegetative stage, in the cultural forms.
5. In cultures, too, chromidial changes occur in the precystic and cystic forms which point towards a specific sexual process. Some of the appearances in these stages are similar to certain forms of the Negri bodies.
6. A culture of a saprophytic ameba (*Ameba limax*) produced the clinical symptoms of bloody dysentery in a kitten. The organism was recovered from the stools, with no morphologic changes in the first cultures isolated.

In summarizing, we may say that the work so far seems to indicate that cultural forms of intestinal amebas do not differ materially from the forms in their natural habitat, and that there are probably several varieties and possibly more than one species, which produce amebic dysentery, as there are undoubtedly several varieties and species of non-pathogenic amebas in the human intestines.

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